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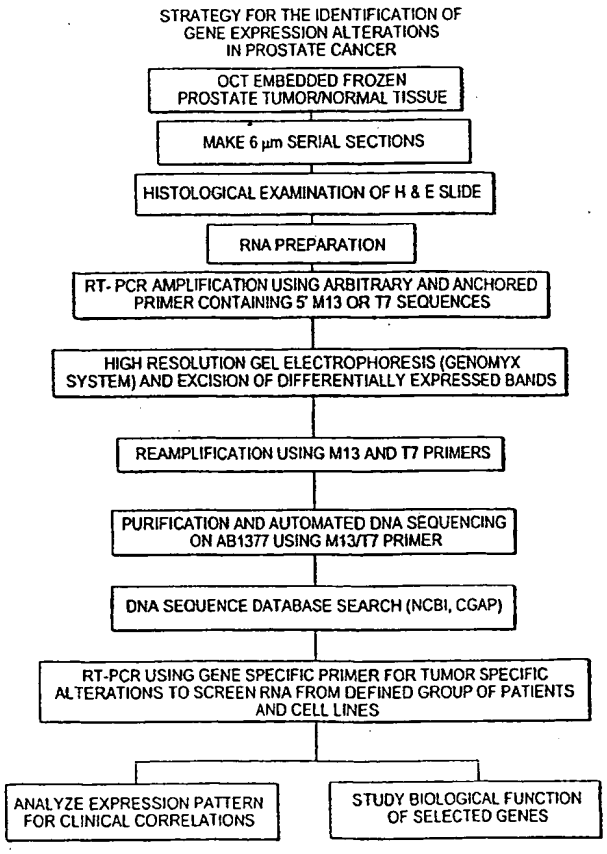
(57) Abstract

A nucleic acid sequence that exhibits prostate-specific expression and overexpression in tumor cells is disclosed. The sequence and fragments thereof are useful for detecting, diagnosing, preventing, and treating prostate cancer and other prostate related diseases. The sequence is also useful for measuring hormone responsiveness of prostate cancer cells.



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PROSTATE-SPECIFIC GENE, PCGEM1, AND METHODS OF USING PCGEM1 TO DETECT, TREAT, AND PREVENT PROSTATE CANCER

CROSS REFERENCE TO RELATED APPLICATIONS

The present application claims the benefit of United States provisional application S.N. 60/126,469, filed March 26, 1999, the entire disclosure of which is relied upon and incorporated by reference.

FIELD OF THE INVENTION

The present invention relates to nucleic acids that are expressed in prostate tissue. More particularly, the present invention relates to the first of a family of novel, androgen-regulated, prostate-specific genes, PCGEM1, that is over-expressed in prostate cancer, and methods of using the PCGEM1 sequence and fragments thereof to measure the hormone responsiveness of prostate cancer cells and to detect, diagnose, prevent and treat prostate cancer and other prostate related diseases.

BACKGROUND

Prostate cancer is the most common solid tumor in American men (1). The wide spectrum of biologic behavior (2) exhibited by prostatic neoplasms poses a difficult problem in predicting the clinical course for the individual patient (3, 4). Public awareness of prostate specific antigen (PSA) screening efforts has led to an increased diagnosis of prostate cancer. The increased diagnosis and greater number of patients presenting with prostate cancer has resulted in wider use of radical prostatectomy for localized disease (5). Accompanying the rise in surgical intervention is the frustrating realization of the inability to predict organ-confined disease and clinical outcome for a given patient (5, 6). Traditional prognostic markers, such as grade, clinical stage, and pretreatment PSA have limited prognostic value for individual men. There is clearly a need to recognize and develop molecular and genetic biomarkers to improve prognostication and the management of patients with clinically localized prostate cancer. As with other common human neoplasia (7), the search for molecular and genetic biomarkers to better define the genesis and progression of prostate cancer is the key focus for cancer research investigations worldwide.

The new wave of research addressing molecular genetic alterations in prostate cancer is primarily due to increased awareness of this disease and the development of

newer molecular technologies. The search for the precursor of prostatic adenocarcinoma has focused largely on the spectrum of microscopic changes referred to as "prostatic intraepithelial neoplasia" (PIN). Bostwick defines this spectrum as a histopathologic continuum that culminates in high grade PIN and early invasive cancer (8). The morphologic and molecular changes include the progressive disruption of the basal cell-layer, changes in the expression of differentiation markers of the prostatic secretory epithelial cells, nuclear and nucleolar abnormalities, increased cell proliferation, DNA content alterations, and chromosomal and allelic losses (8, 9). These molecular and genetic biomarkers, particularly their progressive gain or loss, can be followed to trace the etiology of prostate carcinogenesis. Foremost among these biomarkers would be the molecular and genetic markers associated with histological phenotypes in transition between normal prostatic epithelium and cancer. Most studies so far seem to agree that PIN and prostatic adenocarcinoma cells have a lot in common with each other. The invasive carcinoma more often reflects a magnification of some of the events already manifest in PIN.

Early detection of prostate cancer is possible today because of the widely propagated and recommended blood PSA test that provides a warning signal for prostate cancer if high levels of serum PSA are detected. However, when used alone, PSA is not sufficiently sensitive or specific to be considered an ideal tool for the early detection or staging of prostate cancer (10). Combining PSA levels with clinical staging and Gleason scores is more predictive of the pathological stage of localized prostate cancer (11). In addition, new molecular techniques are being used for improved molecular staging of prostate cancer (12, 13). For instance, reverse transcriptase - polymerase chain reaction (RT-PCR) can measure PSA of circulating prostate cells in blood and bone marrow of prostate cancer patients.

Despite new molecular techniques, however, as many as 25 percent of men with prostate cancer will have normal PSA levels – usually defined as those equal to or below 4 nanograms per milliliter of blood (14). In addition, more than 50 percent of the men with higher PSA levels are actually cancer free (14). Thus, PSA is not an ideal screening tool for prostate cancer. More reliable tumor-specific biomarkers are

needed that can distinguish between normal and hyperplastic epithelium, and the preneoplastic and neoplastic stages of prostate cancer.

Identification and characterization of genetic alterations defining prostate cancer onset and progression is important in understanding the biology and clinical course of the disease. The currently available TNM staging system assigns the original primary tumor (T) to one of four stages (14). The first stage, T1, indicates that the tumor is microscopic and cannot be felt on rectal examination. T2 refers to tumors that are palpable but fully contained within the prostate gland. A T3 designation indicates the cancer has spread beyond the prostate into surrounding connective tissue or has invaded the neighboring seminal vesicles. T4 cancer has spread even further. The TNM staging system also assesses whether the cancer has metastasized to the pelvic lymph nodes (N) or beyond (M). Metastatic tumors result when cancer cells break away from the original tumor, circulate through the blood or lymph, and proliferate at distant sites in the body.

Recent studies of metastatic prostate cancer have shown a significant heterogeneity of allelic losses of different chromosome regions between multiple cancer foci (21-23). These studies have also documented that the metastatic lesion can arise from cancer foci other than dominant tumors (22). Therefore, it is critical to understand the molecular changes which define the prostate cancer metastasis especially when prostate cancer is increasingly detected in early stages (15-21).

Moreover, the multifocal nature of prostate cancer needs to be considered (22-23) when analyzing biomarkers that may have potential to predict tumor progression or metastasis. Approximately 50-60% of patients treated with radical prostatectomy for localized prostate carcinomas are found to have microscopic disease that is not organ confined, and a significant portion of these patients relapse (24). Utilizing biostatistical modeling of traditional and genetic biomarkers such as p53 and *bcl*-2, Bauer et al. (25-26) were able to identify patients at risk of cancer recurrence after surgery. Thus, there is clearly a need to develop biomarkers defining various stages of the prostate cancer progression.

Another significant aspect of prostate cancer is the key role that androgens play in the development of both the normal prostate and prostate cancer. Androgen

ablation, also referred to as "hormonal therapy," is a common treatment for prostate cancer, particularly in patients with metastatic disease (14). Hormonal therapy aims to inhibit the body from making androgens or to block the activity of androgen. One way to block androgen activity involves blocking the androgen receptor; however, that blockage is often only successful initially. For example, 70-80% of patients with advanced disease exhibit an initial subjective response to hormonal therapy, but most tumors progress to an androgen-independent state within two years (16). One mechanism proposed for the progression to an androgen-independent state involves constitutive activation of the androgen signaling pathway, which could arise from structural changes in the androgen receptor protein (16).

As indicated above, the genesis and progression of cancer cells involve multiple genetic alterations as well as a complex interaction of several gene products. Thus, various strategies are required to fully understand the molecular genetic alterations in a specific type of cancer. In the past, most molecular biology studies had focused on mutations of cellular proto-oncogenes and tumor suppressor genes (TSGs) associated with prostate cancer (7). Recently, however, there has been an increasing shift toward the analysis of "expression genetics" in human cancer (27-31), *i.e.*, the under-expression or over-expression of cancer-specific genes. This shift addresses limitations of the previous approaches including: 1) labor intensive technology involved in identifying mutated genes that are associated with human cancer; 2) the limitations of experimental models with a bias toward identification of only certain classes of genes, *e.g.*, identification of mutant *ras* genes by transfection of human tumor DNAs utilizing NIH3T3 cells; and 3) the recognition that the human cancer associated genes identified so far do not account for the diversity of cancer phenotypes.

A number of studies are now addressing the alterations of prostate cancer-associated gene expression in patient specimens (32-36). It is inevitable that more reports on these lines are to follow.

Thus, despite the growing body of knowledge regarding prostate cancer, there is still a need in the art to uncover the identity and function of the genes involved in prostate cancer pathogenesis. There is also a need for reagents and assays to

accurately detect cancerous cells, to define various stages of prostate cancer progression, to identify and characterize genetic alterations defining prostate cancer onset and progression, to detect micro-metastasis of prostate cancer, and to treat and prevent prostate cancer.

SUMMARY OF THE INVENTION

The present invention relates to the identification and characterization of a novel gene, the first of a family of genes, designated PCGEM1, for Prostate Cancer Gene Expression Marker 1. PCGEM1 is specific to prostate tissue, is androgen-regulated, and appears to be over-expressed in prostate cancer. More recent studies associate PCGEM1 cDNA with promoting cell growth. The invention provides the isolated nucleotide sequence of PCGEM1 or fragments thereof and nucleic acid sequences that hybridize to PCGEM1. These sequences have utility, for example, as markers of prostate cancer and other prostate related diseases, and as targets for therapeutic intervention in prostate cancer and other prostate related diseases. The invention further provides a vector that directs the expression of PCGEM1, and a host cell transfected or transduced with this vector.

In another embodiment, the invention provides a method of detecting prostate cancer cells in a biological sample, for example, by using nucleic acid amplification techniques with primers and probes selected to bind specifically to the PCGEM1 sequence. The invention further comprises a method of selectively killing a prostate cancer cell, a method of identifying an androgen responsive cell line, and a method of measuring responsiveness of a cell line to hormone-ablation therapy.

In another aspect, the invention relates to an isolated polypeptide encoded by the PCGEM1 gene or a fragment thereof, and antibodies generated against the PCGEM1 polypeptide, peptides, or portions thereof, which can be used to detect, treat, and prevent prostate cancer.

Additional features and advantages of the invention will be set forth in the description which follows, and in part will be apparent from the description, or may be learned by practice of the invention. The objectives and other advantages of the invention will be realized and attained by the sequences, cells, vectors, and methods

particularly pointed out in the written description and claims herein as well as the appended drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the scheme for the identification of differentially expressed genes in prostate tumor and normal tissues.

Figure 2 depicts a differential display pattern of mRNA obtained from matched tumor and normal tissues of a prostate cancer patient. Arrows indicate differentially expressed cDNAs.

Figure 3 depicts the analysis of PCGEM1 expression in primary prostate cancers.

Figure 4 depicts the expression pattern of PCGEM1 in prostate cancer cell lines.

Figure 5a depicts the androgen regulation of PCGEM1 expression in LNCaP cells, as measured by reverse transcriptase PCR.

Figure 5b depicts the androgen regulation of PCGEM1 expression in LNCaP cells, as measured by Northern blot hybridization.

Figure 6a depicts the prostate tissue specific expression pattern of PCGEM1.

Figure 6b depicts a RNA master blot showing the prostate tissue specificity of PCGEM1.

Figure 7A depicts the chromosomal localization of PCGEM1 by fluorescent in situ hybridization analysis.

Figure 7B depicts a DAPI counter-stained chromosome 2 (left), an inverted DAPI stained chromosome 2 shown as G-bands (center), and an ideogram of chromosome 2 showing the localization of the signal to band 2q32(bar).

Figure 8 depicts a cDNA sequence of PCGEM1 (SEQ ID NO:1).

Figure 9 depicts an additional cDNA sequence of PCGEM1 (SEQ ID NO:2).

Figure 10 depicts the colony formation of NIH3T3 cell lines expressing various PCGEM1 constructs.

Figure 11 depicts the cDNA sequence of the promoter region of PCGEM1 SEQ ID NO:3.

Figure 12 depicts the cDNA of a probe, designated SEQ ID NO:4.

Figure 13 depicts the cDNAs of primers 1-3, designated SEQ ID NOs:5-7, respectively.

Figure 14 depicts the genomic DNA sequence of PCGEM1, designated SEQ ID NO:8.

Figure 15 depicts the structure of the PCGEM1 transcription unit.

Figure 16 depicts a graph of the hypothetical coding capacity of PCGEM1.

Figure 17 depicts a representative example of *in situ* hybridization results showing PCGEM1 expression in normal and tumor areas of prostate cancer tissues.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to PCGEM1, the first of a family of genes, and its related nucleic acids, proteins, antigens, and antibodies for use in the detection, prevention, and treatment of prostate cancer (*e.g.*, prostatic intraepithelial neoplasia (PIN), adenocarcinomas, nodular hyperplasia, and large duct carcinomas) and prostate related diseases (*e.g.*, benign prostatic hyperplasia), and kits comprising these reagents.

Although we do not wish to be limited by any theory or hypothesis, preliminary data suggest that the PCGEM1 nucleotide sequence may be related to a family of non-coding poly A+RNA that may be implicated in processes relating to growth and embryonic development (40-44). Evidence presented herein supports this hypothesis. Alternatively, PCGEM1 cDNA may encode a small peptide.

NUCLEIC ACID MOLECULES

In a particular embodiment, the invention relates to certain isolated nucleotide sequences that are substantially free from contaminating endogenous material. A "nucleotide sequence" refers to a polynucleotide molecule in the form of a separate fragment or as a component of a larger nucleic acid construct. The nucleic acid molecule has been derived from DNA or RNA isolated at least once in substantially pure form and in a quantity or concentration enabling identification, manipulation, and recovery of its component nucleotide sequences by standard biochemical methods

(such as those outlined in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989)).

Nucleic acid molecules of the invention include DNA in both single-stranded and double-stranded form, as well as the RNA complement thereof. DNA includes, for example, cDNA, genomic DNA, chemically synthesized DNA, DNA amplified by PCR, and combinations thereof. Genomic DNA may be isolated by conventional techniques, *e.g.*, using the cDNA of SEQ ID NO:1, SEQ ID NO:2, or suitable fragments thereof, as a probe.

The DNA molecules of the invention include full length genes as well as polynucleotides and fragments thereof. The full length gene may include the N-terminal signal peptide. Although a non-coding role of PCGEM1 appears likely, the possibility of a protein product cannot presently be ruled out. Therefore, other embodiments may include DNA encoding a soluble form, *e.g.*, encoding the extracellular domain of the protein, either with or without the signal peptide.

The nucleic acids of the invention are preferentially derived from human sources, but the invention includes those derived from non-human species, as well.

Preferred Sequences

Particularly preferred nucleotide sequences of the invention are SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO: 8, as set forth in Figures 8, 9, and 14, respectively. Two cDNA clones having the nucleotide sequences of SEQ ID NO:1 and SEQ ID NO:2, and the genomic DNA having the nucleotide sequence of SEQ ID NO: 8, were isolated as described in Example 2.

Thus, in a particular embodiment, this invention provides an isolated nucleic acid molecule selected from the group consisting of (a) the polynucleotide sequence of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO: 8; (b) an isolated nucleic acid molecule that hybridizes to either strand of a denatured, double-stranded DNA comprising the nucleic acid sequence of (a) under conditions of moderate stringency in 50% formamide and about 6X SSC at about 42°C with washing conditions of approximately 60°C, about 0.5X SSC, and about 0.1% SDS; (c) an isolated nucleic acid molecule that hybridizes to either strand of a denatured, double-stranded DNA

comprising the nucleic acid sequence of (a) under conditions of high stringency in 50% formamide and about 6X SSC, with washing conditions of approximately 68°C, about 0.2X SSC, and about 0.1% SDS; (d) an isolated nucleic acid molecule derived by *in vitro* mutagenesis from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:8; (e) an isolated nucleic acid molecule degenerate from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:8 as a result of the genetic code; and (f) an isolated nucleic acid molecule selected from the group consisting of human PCGEM1 DNA, an allelic variant of human PCGEM1 DNA, and a species homolog of PCGEM1 DNA.

As used herein, conditions of moderate stringency can be readily determined by those having ordinary skill in the art based on, for example, the length of the DNA. The basic conditions are set forth by Sambrook et al. *Molecular Cloning: A Laboratory Manual*, 2d ed. Vol. 1, pp. 1.101-104, Cold Spring Harbor Laboratory Press, (1989), and include use of a prewashing solution for the nitrocellulose filters of about 5X SSC, about 0.5% SDS, and about 1.0 mM EDTA (pH 8.0), hybridization conditions of about 50% formamide, about 6X SSC at about 42°C (or other similar hybridization solution, such as Stark's solution, in about 50% formamide at about 42°C), and washing conditions of about 60°C, about 0.5X SSC, and about 0.1% SDS. Conditions of high stringency can also be readily determined by the skilled artisan based on, for example, the length of the DNA. Generally, such conditions are defined as hybridization conditions as above, and with washing at approximately 68°C, about 0.2X SSC, and about 0.1% SDS. The skilled artisan will recognize that the temperature and wash solution salt concentration can be adjusted as necessary according to factors such as the length of the probe.

Additional Sequences

Due to the known degeneracy of the genetic code, wherein more than one codon can encode the same amino acid, a DNA sequence can vary from that shown in SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:8, and still encode PCGEM1. Such variant DNA sequences can result from silent mutations (*e.g.*, occurring during PCR amplification), or can be the product of deliberate mutagenesis of a native sequence.

The invention thus provides isolated DNA sequences of the invention selected from: (a) DNA comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:8; (b) DNA capable of hybridization to a DNA of (a) under conditions of moderate stringency; (c) DNA capable of hybridization to a DNA of (a) under conditions of high stringency; and (d) DNA which is degenerate as a result of the genetic code to a DNA defined in (a), (b), or (c). Such sequences are preferably provided and/or constructed in the form of an open reading frame uninterrupted by internal non-translated sequences, or introns, that are typically present in eukaryotic genes. Sequences of non-translated DNA can be present 5′ or 3′ from an open reading frame, where the same do not interfere with manipulation or expression of the coding region. Of course, should PCGEM1 encode a polypeptide, polypeptides encoded by such DNA sequences are encompassed by the invention. Conditions of moderate and high stringency are described above.

In another embodiment, the nucleic acid molecules of the invention comprise nucleotide sequences that are at least 80% identical to a nucleotide sequence set forth herein. Also contemplated are embodiments in which a nucleic acid molecule comprises a sequence that is at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, or at least 99.9% identical to a nucleotide sequence set forth herein.

Percent identity may be determined by visual inspection and mathematical calculation. Alternatively, percent identity of two nucleic acid sequences may be determined by comparing sequence information using the GAP computer program, version 6.0 described by Devereux et al. (*Nucl. Acids Res.* 12:387, 1984) and available from the University of Wisconsin Genetics Computer Group (UWGCG). The preferred default parameters for the GAP program include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess, *Nucl. Acids Res.* 14:6745, 1986, as described by Schwartz and Dayhoff, eds., *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358, 1979; (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each

gap; and (3) no penalty for end gaps. Other programs used by one skilled in the art of sequence comparison may also be used.

The invention also provides isolated nucleic acids useful in the production of polypeptides. Such polypeptides may be prepared by any of a number of conventional techniques. A DNA sequence of this invention or desired fragment thereof may be subcloned into an expression vector for production of the polypeptide or fragment. The DNA sequence advantageously is fused to a sequence encoding a suitable leader or signal peptide. Alternatively, the desired fragment may be chemically synthesized using known techniques. DNA fragments also may be produced by restriction endonuclease digestion of a full length cloned DNA sequence, and isolated by electrophoresis on agarose gels. If necessary, oligonucleotides that reconstruct the 5′ or 3′ terminus to a desired point may be ligated to a DNA fragment generated by restriction enzyme digestion. Such oligonucleotides may additionally contain a restriction endonuclease cleavage site upstream of the desired coding sequence, and position an initiation codon (ATG) at the N-terminus of the coding sequence.

The well-known polymerase chain reaction (PCR) procedure also may be employed to isolate and amplify a DNA sequence encoding a desired protein fragment. Oligonucleotides that define the desired termini of the DNA fragment are employed as 5′ and 3′ primers. The oligonucleotides may additionally contain recognition sites for restriction endonucleases, to facilitate insertion of the amplified DNA fragment into an expression vector. PCR techniques are described in Saiki et al., *Science* 239:487 (1988); *Recombinant DNA Methodology*, Wu et al., eds., Academic Press, Inc., San Diego (1989), pp. 189-196; and *PCR Protocols: A Guide to Methods and Applications*, Innis et al., eds., Academic Press, Inc. (1990).

USE OF PCGEM1 NUCLEIC ACID OR OLIGONUCLEOTIDES

In a particular embodiment, the invention relates to PCGEM1 nucleotide sequences isolated from human prostate cells, including the complete genomic DNA (Figure 14, SEQ ID NO: 8), and two full length cDNAs: SEQ ID NO:1 (Figure 8) and SEQ ID NO:2 (Figure 9), and fragments thereof. The nucleic acids of the invention, including DNA, RNA, mRNA and oligonucleotides thereof, are useful in a variety of

applications in the detection, diagnosis, prognosis, and treatment of prostate cancer. Examples of applications within the scope of the present invention include, but are not limited to:

- amplifying PCGEM1 sequences;
- detecting a PCGEM1-derived marker of prostate cancer by hybridization with an oligonucleotide probe;
- identifying chromosome 2;
- mapping genes to chromosome 2;
- identifying genes associated with certain diseases, syndromes, or other conditions associated with human chromosome 2;
- constructing vectors having PCGEM1 sequences;
- expressing vector-associated PCGEM1 sequences as RNA and protein;
- detecting defective genes in an individual;
- developing gene therapy;
- developing immunologic reagents corresponding to PCGEM1-encoded products; and
- treating prostate cancer using antibodies, antisense nucleic acids, or other inhibitors specific for PCGEM1 sequences.

Detecting, Diagnosing, and Treating Prostate Cancer

The present invention provides a method of detecting prostate cancer in a patient, which comprises (a) detecting PCGEM1 mRNA in a biological sample from the patient; and (b) correlating the amount of PCGEM1 mRNA in the sample with the presence of prostate cancer in the patient. Detecting PCGEM1 mRNA in a biological sample may include: (a) isolating RNA from said biological sample; (b) amplifying a PCGEM1 cDNA molecule; (c) incubating the PCGEM1 cDNA with the isolated nucleic acid of the invention; and (d) detecting hybridization between the PCGEM1 cDNA and the isolated nucleic acid. The biological sample can be selected from the group consisting of blood, urine, and tissue, for example, from a biopsy. In a preferred embodiment, the biological sample is blood. This method is useful in both the initial diagnosis of prostate cancer, and the later prognosis of disease. This

method allows for testing prostate tissue in a biopsy, and after removal of a cancerous prostate, continued monitoring of the blood for micrometastases.

According to this method of diagnosing and prognosticating prostate cancer in a patient, the amount of PCGEM1 mRNA in a biological sample from a patient is correlated with the presence of prostate cancer in the patient. Those of ordinary skill in the art can readily assess the level of over-expression that is correlated with the presence of prostate cancer.

In another embodiment, this invention provides a vector, comprising a PCGEM1 promoter sequence operatively linked to a nucleotide sequence encoding a cytotoxic protein. The invention further provides a method of selectively killing a prostate cancer cell, which comprises introducing the vector to prostate cancer cells under conditions sufficient to permit selective killing of the prostate cells. As used herein, the phrase "selective killing" is meant to include the killing of at least a cell which is specifically targeted by a nucleotide sequence. The putative PCGEM1 promoter, contained in the 5′ flanking region of the PCGEM1 genomic sequence, SEQ ID NO: 3, is set forth in Figure 11. Applicants envision that a nucleotide sequence encoding any cytotoxic protein can be incorporated into this vector for delivery to prostate tissue. For example, the cytotoxic protein can be ricin, abrin, diphtheria toxin, p53, thymidine kinase, tumor necrosis factor, cholera toxin, *Pseudomonas aeruginosa* exotoxin A, ribosomal inactivating proteins, or mycotoxins such as trichothecenes, and derivatives and fragments (*e.g.*, single chains) thereof.

This invention also provides a method of identifying an androgen-responsive cell line, which comprises (a) obtaining a cell line suspected of being androgen-responsive, (b) incubating the cell line with an androgen; and (c) detecting PCGEM1 mRNA in the cell line, wherein an increase in PCGEM1 mRNA, as compared to an untreated cell line, correlates with the cell line being androgen-responsive.

The invention further provides a method of measuring the responsiveness of a prostatic tissue to hormone-ablation therapy, which comprises (a) treating the prostatic tissue with hormone-ablation therapy; and (b) measuring PCGEM1 mRNA in the prostatic tissue following hormone-ablation therapy, wherein a decrease in

PCGEM1 mRNA, as compared to an untreated cell line, correlates with the cell line responding to hormone-ablation therapy.

In another aspect of the invention, these nucleic acid molecules may be introduced into a recombinant vector, such as a plasmid, cosmid, or virus, which can be used to transfect or transduce a host cell. The nucleic acids of the present invention may be combined with other DNA sequences, such as promoters, polyadenylation signals, restriction enzyme sites, multiple cloning sites, and other coding sequences.

Probes

Among the uses of nucleic acids of the invention is the use of fragments as probes or primers. Such fragments generally comprise at least about 17 contiguous nucleotides of a DNA sequence. The fragment may have fewer than 17 nucleotides, such as, for example, 10 or 15 nucleotides. In other embodiments, a DNA fragment comprises at least 20, at least 30, or at least 60 contiguous nucleotides of a DNA sequence. Examples of probes or primers of the invention include those of SEQ ID NO: 5, SEQ ID NO: 6, and SEQ ID NO: 7, as well as those disclosed in Table I.

Table I

Primer	Sequence (5'→3')	S/AS	Starting Base #	SEQ ID NO.
p413	TGGCAACAGGCAAGCAGAG	S	510	SEQ ID NO: 9
p414	GGCCAAAATAAAACCAAACAT	AS	610	SEQ ID NO: 10
p489	GCAAATATGATTTAAAGATACAAC	S	752	SEQ ID NO: 11
p490	GGTTGTATCTTTAAATCATATTTGC	AS	776	SEQ ID NO: 12
p491	ACTGTCTTTTCATATATTTCTCAATGC	S	559	SEQ ID NO: 13
p517	AAGTAGTAATTTTAAACATGGGAC	AS	1516	SEQ ID NO: 14
p518	TTTTTCAATTAGGCAGCAACC	S	131	SEQ ID NO: 15
p519	GAATTGTCTTTGTGATTGTTTTTAG	S	1338	SEQ ID NO: 16
p560	CAATTCACAAAGACAATTCAGTTAAG	AS	1355	SEQ ID NO: 17
p561	ACAATTAGACAATGTCCAGCTGA	AS	1154	SEQ ID NO: 18
p562	CTTTGGCTGATATCATGAAGTGTC	AS	322	SEQ ID NO: 19
p623	AACCTTTTGCCCTATGCCGTAAC	S	148	SEQ ID NO: 20
p624	GAGACTCCCAACCTGATGATGT	AS	376	SEQ ID NO: 21
p839	GGTCACGTTGAGTCCCAGTG	AS	270	SEQ ID NO: 22

S/AS indicates whether the primer is Sense or AntiSense
Starting Base # indicates the starting base number with respect to the sequence of SEQ ID NO:1.

However, even larger probes may be used. For example, a particularly preferred probe is derived from PCGEM1 (SEQ ID NO: 1) and comprises nucleotides 116 to 1140 of that sequence. It has been designated SEQ ID NO: 4 and is set forth in Figure 12.

When a hybridization probe binds to a target sequence, it forms a duplex molecule that is both stable and selective. These nucleic acid molecules may be readily prepared, for example, by chemical synthesis or by recombinant techniques. A wide variety of methods are known in the art for detecting hybridization, including fluorescent, radioactive, or enzymatic means, or other ligands such as avidin/biotin.

In another aspect of the invention, these nucleic acid molecules may be introduced into a recombinant vector, such as a plasmid, cosmid, or virus, which can be used to transfect or transduce a host cell. The nucleic acids of the present invention may be combined with other DNA sequences, such as promoters, polyadenylation signals, restriction enzyme sites, multiple cloning sites, and other coding sequences.

Because homologs of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 8 from other mammalian species are contemplated herein, probes based on the human DNA sequence of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 8 may be used to screen cDNA libraries derived from other mammalian species, using conventional cross-species hybridization techniques.

In another aspect of the invention, one can use the knowledge of the genetic code in combination with the sequences set forth herein to prepare sets of degenerate oligonucleotides. Such oligonucleotides are useful as primers, *e.g.*, in polymerase chain reactions (PCR), whereby DNA fragments are isolated and amplified. Particularly preferred primers are set forth in Figures 13 and Table I and are designated SEQ ID NOS: 5-7 and 9-22, respectively. A particularly preferred primer pair is p518 (SEQ ID NO: 15) and p839 (SEQ ID NO: 22), which when used in PCR, preferentially amplifies mRNA, thereby avoiding less desirable cross-reactivity with genomic DNA.

Chromosome Mapping

As set forth in Example 3, the PCGEM1 gene has been mapped by fluorescent in situ hybridization to the 2q32 region of chromosome 2 using a bacterial artificial chromosome (BAC) clone containing PCGEU1 genomic sequence. Thus, all or a portion of the nucleic acid molecule of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:8, including oligonucleotides, can be used by those skilled in the art using well-known techniques to identify human chromosome 2, and the specific locus thereof, that contains the PCGEM1 DNA. Useful techniques include, but are not limited to, using the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:2, or SE ID NO:8, or fragments thereof, including oligonucleotides, as a probe in various well-known techniques such as radiation hybrid mapping (high resolution), *in situ* hybridization to chromosome spreads (moderate resolution), and Southern blot hybridization to hybrid cell lines containing individual human chromosomes (low resolution).

For example, chromosomes can be mapped by radiation hybridization. First, PCR is performed using the Whitehead Institute/MIT Center for Genome Research Genebridge4 panel of 93 radiation hybrids

(http://www-genome.wi.mit.edu/ftp/distribution/ human_STS_releases/july97/rhmap/genebridge4.html). Primers are used which lie within a putative exon of the gene of interest and which amplify a product from human genomic DNA, but do not amplify hamster genomic DNA. The results of the PCRs are converted into a data vector that is submitted to the Whitehead/MIT Radiation Mapping site on the internet (http://www-seq.wi.mit.edu). The data is scored and the chromosomal assignment and placement relative to known Sequence Tag Site (STS) markers on the radiation hybrid map is provided. (The following web site provides additional information about radiation hybrid mapping: http://www-genome.wi.mit.edu/ftp/distribution/human_STS_releases/july97/ 07-97.INTRO.html).

Identifying Associated Diseases

As noted above, PCGEM1 has been mapped to the 2q32 region of chromosome 2. This region is associated with specific diseases, which include but are not limited to diabetes mellitus (insulin dependent), and T cell leukemia/lymphoma. Thus, the nucleic acids of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO:8, or fragments thereof, can be used by one skilled in the art using well-known techniques to analyze abnormalities associated with gene mapping to chromosome 2. This enables one to distinguish conditions in which this marker is rearranged or deleted. In addition, nucleotides of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:8, or fragments thereof, can be used as a positional marker to map other genes of unknown location.

The DNA may be used in developing treatments for any disorder mediated (directly or indirectly) by defective, or insufficient amounts of PCGEM1, including prostate cancer. Disclosure herein of native nucleotide sequences permits the detection of defective genes, and the replacement thereof with normal genes. Defective genes may be detected in *in vitro* diagnostic assays, and by comparison of a native nucleotide sequence disclosed herein with that of a gene derived from a person suspected of harboring a defect in this gene.

Sense-Antisense

Other useful fragments of the nucleic acids include antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target mRNA (sense) or DNA (antisense) sequences. Antisense or sense oligonucleotides, according to the present invention, comprise a fragment of DNA (SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:8). Such a fragment generally comprises at least about 14 nucleotides, preferably from about 14 to about 30 nucleotides. The ability to derive an antisense or a sense oligonucleotide, based upon a cDNA sequence encoding a given protein is described in, for example, Stein and Cohen (*Cancer Res.* 48:2659, 1988) and van der Krol et al. (*BioTechniques* 6:958, 1988).

The biologic activity of PCGEM1 in assay cells and the over expression of PCGEM1 in prostate cancer tissues suggest that elevated levels of PCGEM1 promote prostate cancer cell growth. Thus, the antisense oligonucleotides to PCGEM1 may be used to reduce the expression of PCGEM1 and, consequently, inhibit the growth of the cancer cells.

Binding of antisense or sense oligonucleotides to target nucleic acid sequences results in the formation of duplexes. The antisense oligonucleotides thus may be used to block expression of proteins or to inhibit the function of RNA. Antisense or sense oligonucleotides further comprise oligonucleotides having modified sugar-phosphodiester backbones (or other sugar linkages, such as those described in WO91/06629) and wherein such sugar linkages are resistant to endogenous nucleases. Such oligonucleotides with resistant sugar linkages are stable *in vivo* (*i.e.*, capable of resisting enzymatic degradation) but retain sequence specificity to be able to bind to target nucleotide sequences.

Other examples of sense or antisense oligonucleotides include those oligonucleotides which are covalently linked to organic moieties, such as those described in WO 90/10448, and other moieties that increases affinity of the oligonucleotide for a target nucleic acid sequence, such as poly-(L-lysine). Further still, intercalating agents, such as ellipticine, and alkylating agents or metal complexes may be attached to sense or antisense oligonucleotides. Such modifications may

modify binding specificities of the antisense or sense oligonucleotide for the target nucleotide sequence.

Antisense or sense oligonucleotides may be introduced into a cell containing the target nucleic acid sequence by any gene transfer method, including, for example, lipofection, $CaPO_4$-mediated DNA transfection, electroporation, or by using gene transfer vectors such as Epstein-Barr virus or adenovirus.

Sense or antisense oligonucleotides also may be introduced into a cell containing the target nucleotide sequence by formation of a conjugate with a ligand binding molecule, as described in WO 91/04753. Suitable ligand binding molecules include, but are not limited to, cell surface receptors, growth factors, other cytokines, or other ligands that bind to cell surface receptors. Preferably, conjugation of the ligand binding molecule does not substantially interfere with the ability of the ligand binding molecule to bind to its corresponding molecule or receptor, or block entry of the sense or antisense oligonucleotide or its conjugated version into the cell.

Alternatively, a sense or an antisense oligonucleotide may be introduced into a cell containing the target nucleic acid sequence by formation of an oligonucleotide-lipid complex, as described in WO 90/10448. The sense or antisense oligonucleotide-lipid complex is preferably dissociated within the cell by an endogenous lipase.

POLYPEPTIDES AND FRAGMENTS THEREOF

The invention also encompasses polypeptides and fragments thereof in various forms, including those that are naturally occurring or produced through various techniques such as procedures involving recombinant DNA technology. Such forms include, but are not limited to, derivatives, variants, and oligomers, as well as fusion proteins or fragments thereof.

The polypeptides of the invention include full length proteins encoded by the nucleic acid sequences set forth above. The polypeptides of the invention may be membrane bound or they may be secreted and thus soluble. The invention also includes the expression, isolation and purification of the polypeptides and fragments of the invention, accomplished by any suitable technique.

The following examples further illustrate preferred aspects of the invention.

EXAMPLE 1: Differential Gene Expression Analysis in Prostate Cancer

Using the differential display technique, we identified a novel gene that is over-expressed in prostate cancer cells. Differential display provides a method to separate and clone individual messenger RNAs by means of the polymerase chain reaction, as described in Liang et al., *Science*, 257:967-71 (1992), which is hereby incorporated by reference. Briefly, the method entails using two groups of oligonucleotide primers. One group is designed to recognize the polyadenylate tail of messenger RNAs. The other group contains primers that are short and arbitrary in sequence and anneal to positions in the messenger RNA randomly distributed from the polyadenylate tail. Products amplified with these primers can be differentiated on a sequencing gel based on their size. If different cell populations are amplified with the same groups of primers, one can compare the amplification products to identify differentially expressed RNA sequences.

Differential display ("DD") kits from Genomyx (Foster City, California) were used to analyze differential gene expression. The steps of the differential display technique are summarized in Figure 1. Histologically well defined matched tumor and normal prostate tissue sections containing approximately similar proportions of epithelial cells were chosen from individual prostate cancer patients.

Genomic DNA-free total RNA was extracted from this enriched pool of cells using RNAzol B (Tel-Test, Inc., Friendswood, TX) according to manufacturer's protocol. The epithelial nature of the RNA source was further confirmed using cytokeratin 18 expression (45) in reverse transcriptase-polymerase chain reaction (RT-PCR) assays. Using arbitrary and anchored primers containing 5′ M13 or T7 sequences (obtained from Biomedical Instrumentation Center, Uniformed Services University of the Health Sciences, Bethesda), the isolated DNA-free total RNA was amplified by RT-PCR which was performed using ten anchored antisense primers and four arbitrary sense primers according to the protocol provided by Hieroglyph™ RNA Profile Kit 1 (Genomyx Corporation, CA). The cDNA fragments produced by the RT-PCR assay were analyzed by high resolution gel electrophoresis, carried out by

using Genomyx™ LR DNA sequencer and LR-Optimized™ HR-1000™ gel formulations (Genomyx Corporation, CA).

A partial DD screening of normal/tumor tissues revealed 30 differentially expressed cDNA fragments, with 53% showing reduced or no expression in tumor RNA specimens and 47% showing over expression in tumor RNA specimen (Figure 2). These cDNAs were excised from the DD gels, reamplified using T7 and M13 primers and the RT PCR conditions recommended in Hieroglyph™ RNA Profile Kit-1 (Genomyx Corp., CA), and sequenced. The inclusion of T7 and M13 sequencing primers in the DD primers allowed rapid sequencing and orientation of cDNAs (Figure 1).

All the reamplified cDNA fragments were purified by Centricon-c-100 system (Amicon, USA). The purified fragments were sequenced by cycle sequencing and DNA sequence determination using an ABI 377 DNA sequencer. Isolated sequences were analyzed for sequence homology with known sequences by running searches through publicly available DNA sequence databases, including the National Center for Biotechnology Information and the Cancer Genome Anatomy Project. Approximately two-thirds of these cDNA sequences exhibited homology to previously described DNA sequences/genes e.g., ribosomal proteins, mitochondrial DNA sequences, growth factor receptors, and genes involved in maintaining the redox state in cells. About one-third of the cDNAs represented novel sequences, which did not exhibit similarity to the sequences available in publicly available databases. The PCGEM1 fragment, obtained from the initial differential display screening represents a 530 base pair (nucleotides 410 to 940 of SEQ ID NO: 1) cDNA sequence which, in initial searches, did not exhibit any significant homology with sequences in the publicly available databases. Later searching of the high throughput genome sequence (HTGS) database revealed perfect homology to a chromosome 2 derived uncharacterized, unfinished genomic sequence (accession # AC 013401).

EXAMPLE 2: Characterization of Full Length PCGEM1 cDNA Sequence

The full length of PCGEM1 was obtained by 5′ and 3′ RACE/PCR from the original 530 bp DD product (nucleotides 410 to 940 of PCGEM1 cDNA SEQ ID

NO:1) using a normal prostate cDNA library in lambda phage (Clontech, CA). The RACE/PCR products were directly sequenced. Lasergene and MacVector DNA analysis software were used to analyze DNA sequences and to define open reading frame regions. We also used the original DD product to screen a normal prostate cDNA library. Three overlapping cDNA clones were identified.

Sequencing of the cDNA clones was performed on an ABI-310 sequence analyzer and a new dRhodamine cycle sequencing kit (PE-Applied Biosystem, CA). The longest PCGEM1 cDNA clone, SEQ ID NO:1 (Figure 8), revealed 1643 nucleotides with a potential polyadenylation site, ATTAAA, close to the 3′ end followed by a poly (A) tail. As noted above, although initial searching of PCGEM1 gene in publically available DNA databases (e.g., National Center for Biotechnology Information) using the BLAST program did not reveal any homology, a recent search of the HTGS database revealed perfect homology of PCGEM1 (using cDNA of SEQ ID NO: 1) to a chromosome 2 derived uncharacterized, unfinished genomic sequence (accession # AC 013401). One of the cDNA clones, SEQ ID NO:2 (Figure 9), contained a 123 bp insertion at 278, and this inserted sequence showed strong homology (87%) to Alu sequence. It is likely that this clone represented the premature transcripts. Sequencing of several clones from RT-PCR further confirmed the presence of the two forms of transcripts.

Sequence analysis did not reveal any significant long open reading frame in both strands. The longest ORF in the sense strand was 105 nucleotides (572-679) encoding 35 amino acid peptides. However, the ATG was not in a strong context of initiation. Although we could not rule out the coding capacity for a very small peptide, it is possible that PCGEM1 may function as a non-coding RNA.

The sequence of PCGEM1 cDNA has been verified by several approaches including characterization of several clones of PCGEM1 and analysis of PCGEM1 cDNAs amplified from normal prostate tissue and prostate cancer cell lines. We have also obtained the genomic clones of PCGEM1, which has helped to confirm the PCGEM1 cDNA sequence. The complete genomic DNA sequence of PCGEM1 (SEQ ID NO:8) is shown in Figure 14. In Figure 14 (and in the accompanying Sequence Listing), "Y" represents any one of the four nucleotide bases, cylosine,

thymine, adenine, or guanine. Comparison of the cDNA and genomic sequences revealed the organization of the PCGEM1 transcription unit from three exons (Figure 15: E, Exon; B: BamHI; H: HindIII; X: XbaI; R: EcoRI).

EXAMPLE 3: Mapping the Location of PCGEM1

Using fluorescent *in situ* hybridization and the PCGEM1 genomic DNA as a probe, we mapped the location of PCGEM1 on chromosome 2q to specific region 2q32 (Figure 7A). Specifically, a Bacterial Artificial Chromosome (BAC) clone containing the PCGEM1 genomic sequence was isolated by custom services of Genome Systems (St. Louis, Mo). PCGEM1-Bac clone 1 DNA was nick translated using spectrum orange (Vysis) as a direct label and flourescent *in situ* hybridization was done using this probe on normal human male metaphase chromosome spreads. Counterstaining was done and chromosomal localization was determined based on the G-band analysis of inverted 4′,6-diamidino-2-phenylindole (DAPI) images. (Figure 7B: a DAPI counter-stained chromosome 2 is shown on the left; an inverted DAPI stained chromosome 2 shown as G-bands is shown in the center; an ideogram of chromosome 2 showing the localization of the signal to band 2q32(bar) is shown on the right.) NU200 image acquisition and registration software was used to create the digital images. More than 20 metaphases were analyzed.

EXAMPLE 4: Analysis of PCGEM1 Gene Expression in Prostate Cancer

To further characterize the tumor specific expression of the PCGEM1 fragment, and also to rule out individual variations of gene expression alterations commonly observed in tumors, the expression of the PCGEM1 fragment was evaluated on a test panel of matched tumor and normal RNAs derived from the microdissected tissues of twenty prostate cancer patients.

Using the PCGEM1 cDNA sequence (SEQ ID NO:1), specific PCR primers (Sense primer 1 (SEQ ID NO: 5): 5′ TGCCTCAGCCTCCCAAGTAAC 3′ and Antisense primer 2 (SEQ ID NO: 6): 5′ GGCCAAAATAAAACCAAACAT 3′) were designed for RT-PCR assays. Radical prostatectomy derived OCT compound (Miles Inc. Elkhart, IN) embedded fresh frozen normal and tumor tissues from prostate

cancer patients were characterized for histopathology by examining hematoxylin and eosin stained sections (46). Tumor and normal prostate tissues regions representing approximately equal number of epithelial cells were dissected out of frozen sections. DNA-free RNA was prepared from these tissues and used in RT-PCR analysis to detect PCGEM1 expression. One hundred nanograms of total RNA was reverse transcribed into cDNA using RT-PCR kit (Perkin-Elmer, Foster, CA). The PCR was performed using Amplitaq Gold from Perkin-Elmer (Foster, CA). PCR cycles used were: 95°C for 10 minutes, 1 cycle; 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, 42 cycles, and 72°C for 5 minutes, 1 cycle followed by a 4°C storage. Epithelial cell-associated cytokeratin 18 was used as an internal control.

RT-PCR analysis of microdissected matched normal and tumor tissue derived RNAs from 23 CaP patients revealed tumor associated overexpression of PCGEM1 in 13 (56%) of the patients (Figure 5). Six of twenty-three (26%) patients did not exhibit detectable PCGEM1 expression in either normal or tumor tissue derived RNAs. Three of twenty-three (13%) tumor specimens showed reduced expression in tumors. One of the patients did not exhibit any change. Expression of housekeeping genes, cytokeratin-18 (Figure 3) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (data not shown) remained constant in tumor and normal specimens of all the patients (Figure 3). These results were further confirmed by another set of PCGEM1 specific primers (Sense Primer 3 (SEQ ID NO: 7): 5′ TGGCAACAGGCAAGCAGAG 3′ and Antisense Primer 2 (SEQ ID NO: 6): 5′ GGCCAAAATAAAACCAAACAT 3′). Four of 16 (25%) patients did not exhibit detectable PCGEM1 expression in either normal or tumor tissue derived RNAs. Two of 16 (12.5%) tumor specimens showed reduced expression in tumors. These results of PCGEM1 expression in tumor tissues could be explained by the expected individual variations between tumors of different patients. Most importantly, initial DD observations were confirmed by showing that 45% of patients analyzed did exhibit over expression of PCGEM1 in tumor prostate tissues when compared to corresponding normal prostate tissue of the same individual.

EXAMPLE 5: *In situ* Hybridization

In situ hybridization was performed essentially as described by Wilkinson and Green (48). Briefly, OCT embedded tissue slides stored at -80°C were fixed in 4% PFA (paraformaldehyde), digested with proteinase K and then again fixed in 4% PFA. After washing in PBS, sections were treated with 0.25% acetic anhydride in 0.1M triethanolamine, washed again in PBS, and dehydrated in a graded ethanol series. Sections were hybridized with ^{35}S-labeled riboprobes at 52°C overnight. After washing and RNase A treatment, sections were dehydrated, dipped into NTB-2 emulsion and exposed for 11 days at 4°C. After development, slides were lightly stained with hematoxylin and mounted for microscopy. In each section, PCGEM1 expression was scored as percentage of cells showing ^{35}S signal: 1+, 1-25%; 2+, 25-50%; 3+, 50-75%, 4+, 75-100%.

Paired normal (benign) and tumor specimens from 13 patients were tested using *in situ* hybridization. A representative example is shown in Figure 17. In 11 cases (84%) tumor associated elevation of PCGEM1 expression was detected. In 5 of these 11 patients the expression of PCGEM1 increased to 1+ in the tumor area from an essentially undetectable level in the normal area (on the 0 to 4+ scale). Tumor specimens from 4 of 11 patients scored between 2+ (example shown in Figure 17B) and 4+. Two of 11 patients showed focal signals with 3+ score in the tumor area, and one of these patients had similar focal signal (2+) in an area pathologically designated as benign. In the remaining 2 of the 13 cases there was no detectable signal in any of the tissue areas tested. The results indicate that PCGEM1 expression appears to be restricted to glandular epithelial cells. (Figure 17 shows an example of *in situ* hybridization of ^{35}S labeled PCGEM1 riboprobe to matched normal (A) versus tumor (B) sections of prostate cancer patients. The light gray areas are hematoxylin stained cell bodies, the black dots represent the PCGEM1 expression signal. The signal is background level in the normal (A), 2+ level in the tumor (B) section. The magnification is 40x.)

EXAMPLE 6: PCGEM1 Gene Expression in Prostate Tumor Cell Lines

PCGEM1 gene expression was also evaluated in established prostate cancer cell lines: LNCaP, DU145, PC3 (all from ATCC), DuPro (available from Dr. David Paulson, Duke University, Durham, NC), and an E6/E7 - immortalized primary prostate cancer cell line, CPDR1 (47). CPDR1 is a primary CaP derived cell line immortalized by retroviral vector, LXSN 16 E6 E7, expressing E6 and E7 gene of the human papilloma virus 16. LNCaP is a well studied, androgen-responsive prostate cancer cell line, whereas DU145, PC3, DuPro and CPDR1 are androgen-independent and lack detectable expression of the androgen receptor. Utilizing the RT-PCR assay described above, PCGEM1 expression was easily detectable in LNCaP (Figure 4). However, PCGEM1 expression was not detected in prostate cancer cell lines DU145, PC3, DuPro and CPDR. Thus, PCGEM1 was expressed in the androgen-responsive cell line but not in the androgen-independent cell lines. These results indicate that hormones, particularly androgen, may play a key role in regulating PCGEM1 expression in prostate cancer cells. In addition, the results suggest that PCGEM1 expression may be used to distinguish between hormone responsive tumor cells and more aggressive hormone refractory tumor cells.

To test if PCGEM1 expression is regulated by androgens, we performed experiments evaluating PCGEM1 expression in LNCaP cells (ATCC) cultured with and without androgens. Total RNA from LNCaP cells, treated with synthetic androgen R1881 obtained from (DUPONT, Boston, MA), were analyzed for PCGEM1 expression. Both RT-PCR analysis (Figure 5a) and Northern blot analysis (Figure 5b) were conducted as follows.

LNCaP cells were maintained in RPMI 1640 (Life Technologies, Inc., Gaithersburg, MD) supplemented with 10% fetal bovine serum (FBS, Life Technologies, Inc., Gaithersburg, MD) and experiments were performed on cells between passages 20 and 35. For the studies of NKX3.1 gene expression regulation, charcoal/dextran stripped androgen-free FBS (cFBS, Gemini Bio-Products, Inc., Calabasas, CA) was used. LNCaP cells were cultured first in RPMI 1640 with 10% cFBS for 4 days and then stimulated with a non-metabolizable androgen analog R1881 (DUPONT, Boston, MA) at different concentrations for different times as

shown in Figure 5A. LNCaP cells identically treated but without R1881 served as control. Poly A+ RNA derived from cells treated with/without R1881 was extracted at indicated time points with RNAzol B (Tel-Test, Inc, TX) and fractionated (2μg/lane) by running on 1% formaldehyde-agarose gel and transferred to nylon membrane. Northern blots were analyzed for the expression of PCGEM1 using the nucleic acid molecule set forth in SEQ ID NO: 4 as a probe. The RNA from LNCaP cells treated with R1881 and RNA from control LNCaP cells were also analyzed by RT-PCR assays as described in Example 4.

As set forth in Figures 5a and 5b, PCGEM1 expression increases in response to androgen treatment. This finding further supports the hypothesis that the PCGEM1 expression is regulated by androgens in prostate cancer cells.

EXAMPLE 7: Tissue Specificity of PCGEM1 Expression

Multiple tissue Northern blots (Clontech, CA) conducted according to the manufacturer's directions revealed prostate tissue-specific expression of PCGEM1. Polyadenylate RNAs of 23 different human tissues (heart, brain, placenta, lung, liver skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, peripheral blood, stomach, thyroid, spinal cord, lymph node, trachea, adrenal gland and bone marrow) were probed with the 530 base pair PCGEM1 cDNA fragment (nucleotides 410 to 940 of SEQ ID NO:1). A 1.7 kilobase mRNA transcript hybridized to the PCGEM1 probe in prostate tissue (Figure 6a). Hybridization was not observed in any of the other human tissues (Figure 6a). Two independent experiments revealed identical results.

Additional Northern blot analyses on an RNA master blot (Clontech, CA) conducted according to the manufacturer's directions confirm the prostate tissue specificity of the PCGEM1 gene (Figure 6b). Northern blot analyses reveal that the prostate tissue specificity of PCGEM1 is comparable to the well known prostate marker PSA (77mer oligo probe) and far better than two other prostate specific genes PSMA (234 bp fragment from PCR product) and NKX3.1 (210 bp cDNA). For instance, PSMA is expressed in the brain (37) and in the duodenal mucosa and a subset of proximal renal tubules (38). While NKX3.1 exhibits high levels of

expression in adult prostate, it is also expressed in lower levels in testis tissue and several other tissues (39).

EXAMPLE 8: Biologic functions of the PCGEM1

The tumor associated PCGEM1 overexpression suggested that the increased expression of PCGEM1 may favor tumor cell proliferation. NIH3T3 cells have been extensively used to define cell growth promoting functions associated with a wide variety of genes (40-44). Utilizing pcDNA3.1/Hygro(+/-)(Invitrogen, CA), PCGEM1 expression vectors were constructed in sense and anti-sense orientations and were transfected into NIH3T3 cells, and hygromycin resistant colonies were counted 2-3 weeks later. Cells transfected with PCGEM1 sense construct formed about 2 times more colonies than vector alone in three independent experiments (Figure 10). The size of the colonies in PCGEM1 sense construct transfected cells were significantly larger. No appreciable difference was observed in the number of colonies between anti-sense PCGEM1 constructs and vector controls. These promising results document a cell growth promoting/cell survival function(s) associated with PCGEM1.

The function of PCGEM1, however, does not appear to be due to protein expression. To assess this hypothesis, we used the TestCode program (GCG Wisconsin Package, Madison, WI), which identifies potential protein coding sequences of longer than 200 bases by measuring the non-randomness of the composition at every third base, independently from the reading frames. Analysis of the PCGEM1 cDNA sequence revealed that, at greater than 95% confidence level, the sequence does not contain any region with protein coding capacity (Figure 16A). Similar results were obtained when various published non-coding RNA sequences were analyzed with the TestCode program (data not shown), while known protein coding regions of similar size i.e., alpha actin (Figure 16B) can be detected with high fidelity. (In Figure 16, evaluation of the coding capacity of the PCGEM1 (A) and the human alpha actin (B), is performed independently from the reading frame, by using the TestCode program. The number of base pairs is indicated on the X- axis, the TestCode values are shown on the Y-axis. Regions of longer than 200 base pairs

above the upper line (at 9.5 value) are considered coding, under the lower line (at 7.3 value) are considered non-coding, at a confidence level greater than 95%.)

The Codon Preference program (GCG Wisconsin Package, Madison, WI), which locates protein coding regions in a reading frame specific manner further suggested the absence of protein coding capacity in the PCGEM1 gene (see www.cpdr.org). *In vitro* transcription/translation of PCGEM1 cDNA did not produce a detectable protein/peptide. Although we can not unequivocally rule out the possibility that PCGEM1 codes for a short unstable peptide, at this time both experimental and computational approaches strongly suggest that PCGEM1 cDNA does not have protein coding capacity. (It should be recognized that conclusions regarding the role of PCGEM1 are speculative in nature, and should not be considered limiting in any way.

The most intriguing aspect of PCGEM1 characterization has been its apparent lack of protein coding capacity. Although we have not completely ruled out the possibility that PCGEM1 codes for a short unstable peptide, careful sequencing of PCGEM1 cDNA and genomic clones, computational analysis of PCGEM1 sequence, and *in vitro* transcription/translation experiments (data not shown) strongly suggest a non-coding nature of PCGEM1. It is interesting to note that an emerging group of novel mRNA-like non-coding RNAs are being discovered whose function and mechanisms of action remain poorly understood (49). Such RNA molecules have also been termed as "RNA riboregulators" because of their function(s) in development, differentiation, DNA damage, heat shock responses and tumorigenesis (40-42, 50). In the context of tumorigenesis, the *H19, His*-1 and *Bic* genes code for functional non-coding mRNAs (50). In addition, a recently reported prostate cancer associated gene, DD3 also appears to exhibit a tissue specific non-coding mRNA (51). In this regard it is important to point out that PCGEM1 and DD3 may represent a new class of prostate specific genes. The recent discovery of a steroid receptor co-activator as an mRNA, lacking protein coding capacity further emphasizes the role of RNA riboregulators in critical biochemical function(s) (52). Our preliminary results showed that PCGEM1 expression in NIH3T3 cells caused a significant increase in the size of colonies in a colony forming assay and suggests that PCGEM1 cDNA confers

cell proliferation and/or cell survival function(s). Elevated expression of PCGEM1 in prostate cancer cells may represent a gain in function favoring tumor cell proliferation/survival. On the basis of our first characterization of PCGEM1gene, we propose that PCGEM1 belongs to a novel class of prostate tissue specific genes with potential functions in prostate cell biology and the tumorigenesis of the prostate gland.

In summary, utilizing surgical specimens and rapid differential display technology, we have identified candidate genes of interest with differential expression profile in prostate cancer specimens. In particular, we have identified a novel nucleotide sequence, PCGEM1, with no match in the publicly available DNA databases (except for the homology shown in the high throughput genome sequence database, discussed above). A PCGEM1 cDNA fragment detected a 1.7 kb mRNA on Northern blots with selective expression in prostate tissue. Furthermore, this gene was found to be up-regulated by the synthetic androgen, R1881. Careful analysis of microdissected matched tumor and normal tissues further revealed PCGEM1 over-expression in a significant percentage of prostate cancer specimens. Thus, we have provided a gene with broad implications for the diagnosis, prevention, and treatment of prostate cancer.

The specification is most thoroughly understood in light of the teachings of the references cited within the specification which are hereby incorporated by reference. The embodiments within the specification provide an illustration of embodiments of the invention and should not be construed to limit the scope of the invention. The skilled artisan readily recognizes that many other embodiments are encompassed by the invention.

REFERENCES

1. Parker SL, Tong T, Bolden S, and Wingo PA: Cancer statistics. *CA Cancer J. Clin.*, 46:5-27, 1996.

2. Visakorpi T, Kallioniemi OP, Koivula T and Isola J: New prognostic factors in prostate carcinoma. *Eur. Uro.*, 24:438-449, 1993.

3. Mostofi FK: Grading of prostate carcinoma. *Cancer Chemothera Rep.*, 59:111, 1975.

4. Lu-Yao GL, McLerran D, Wasson J, Wennberg JE: An assessment of radical prostatectomy. Time trends, Geographical Variations and Outcomes. *JAMA*, 269:2633-2636, 1993.

5. Partin AW and Oesterling JE: The clinical usefulness of prostate-specific antigen: update 1994, *J. Urol.*, 152:1358-1368, 1994.

6. Wasson JH, Cushman CC, Bruskewitz RC, Littenberg B, Mulley AG, and Wennberg JE: A structured literature review of treatment for localized CaP. *Arch. Fam. Med.*, 2:487-493, 1993.

7. Weinberg RA: How cancer arises. *Sci. Amer.*, 9, 62-70, 1996.

8. Bostwick DG: High grade prostatic intraepithelial neoplasia: The most likely precursor of prostate cancer. *Cancer*, 75:1823-1836, 1995.

9. Bostwick DG, Pacelli A, Lopez-Beltran A: Molecular Biology of Prostatic Intraepithelial Neoplasia. *The Prostate*, 29:117-134, 1996.

10. Pannek J, Partin AW: Prostate specific antigen: What's new in 1997. *Oncology*, 11:1273-1278, 1997.

11. Partin AW, Kattan MW, Subong EN, Walsh PC, Wojno KJ, Oesterling JE, Scardino PT, Pearson JD: Combination of prostate specific antigen, clinical stage, and Gleason score to predict pathological stage of localized prostate cancer. A multi-institutional update. *JAMA*, 277:1445-1451, 1997.

12. Gomella LG, Raj GV, Moreno JG: Reverse transcriptase polymerase chain reaction for prostate specific antigen in management of prostate cancer. *J. Urol.*, 158:326-337, 1997.

13. Gao CL, Dean RC, Pinto A, Mooneyhan R, Connelly RR, McLeod DG, Srivastava, S, Moul JW: Detection of PSA-expressing prostatic cells in bone marrow of radical prostatectomy patients by sensitive reverse transcriptase-polymerase chain reaction (RT-PCR). 1998 International Symposium on Biology of Prostate growth, National Institutes of Health, p. 83, 1998.

14. Garnick MB, Fair WR: Prostate cancer. Sci. Amer., 75-83, 1998.

15. Moul JW, Gaddipati J, and Srivastava S: 1994. Molecular biology of CaP. Oncogenes and tumor suppressor genes. Current Clinical Oncology: CaP. (Eds. Dawson, N.A. and Vogelzang, N.J.), Wiley-Liss Publications, 19-46.

16. Lalani E-N, Laniado ME and Abel PD: Molecular and cellular biology of prostate cancer. *Cancer and Mets. Rev.* 16:29-66, 1997.

17. Shi XB, Gumerlock PH, deVere White RW: Molecular Biology of CaP *World J. Urol*; 14, 318-328, 1996.

18. Heidenberg HB, Bauer JJ, McLeod DG, Moul JW and Srivastava S: The role of p53 tumor suppressor gene in CaP: a possible biomarker? *Urology*, 48:971-979, 1996.

19. Bova GS and Issacs WB: Review of allelic loss and gain in prostate cancer. *World J Urol.*, 14:338-346, 1996.

20. Issacs WB and Bova GS: Prostate Cancer: The Genetic Basis of Human Cancer. Eds. Vogelstein B, and Kinzler KW, McGraw-Hill Companies, Inc., pp. 653-660, 1998.

21. Srivastava S and Moul JW: Molecular Progression of Prostate Cancer. Advances in Oncobiology. (In Press) 1998.

22. Sakr WA, Macoska JA, Benson P, Benson DJ, Wolman SR, Pontes JE, and Crissman: Allelic loss in locally metastatic, multi-sampled prostate cancer. *Cancer Res.*, 54:3273-3277, 1994.

23. Mirchandani D, Zheng J, Miller GL, Ghosh AK, Shibata DK, Cote RJ and Roy-Burman P: Heterogeneity in intratumor distribution of p53 mutations in human prostate cancer. *Am. J. Path.* 147:92-101, 1995.

24. Bauer JJ, Moul JW, and McLeod DG: CaP: Diagnosis, treatment, and experience at one tertiary medical center, 1989-1994. *Military Medicine*, 161:646-653,1996.

25. Bauer JJ, Connelly RR, Sesterhenn IA, Bettencourt MC, McLeod DG, Srivastava S, Moul JW: Biostatistical modeling using traditional variables and genetic biomarkers predicting the risk of prostate cancer recurrence after radical prostatectomy. *Cancer*, 79:952-962, 1997.

26. Bauer JJ, Connelly RR, Sesterhenn IA, DeAusen JD, McLeod DG, Srivastava S, Moul JW: Biostatistical modeling using traditional preoperative and pathological prognostic variables in the selection of men at high risk of disease recurrence after radical prostatectomy. *J. Urol.*, 159(3):929-933, 1998

27. Sager R: Expression genetics in cancer: Shifting the focus from DNA to RNA. *Proc Natl. Acad. Sci. USA*, 94:952-957, 1997

28. Strausberg RL, Dahl CA, and Klausner RD: New opportunities for uncovering the molecular basis of cancer. *Nature Genetics*, 15:415-16, 1997.

29. Liang, Peng, and Pardec AB: Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. *Science* 257:967-971, 1992.

30. Velculescu VE, Zhang L, Vogelstein B, and Kinzler KW: Serial analysis of gene expression. *Science*, 270:484-487, 1995.

31. Chena M, Shalon DS, Davis RW, and Brown PO: Quantitative monitoring of gene expression patterns with a complementary DNA microarrays. *Science*, 270:467-470, 1995.

32. Liu AY, Corey E, Vessella RL, Lange PH, True LD, Huang GM, Nelson PS and Hood L: Identification of differentially expressed prostate genes: Increased expression of transcription factor ETS-2 in prostate cancer. *The Prostate* 30:145-153, 1997.

33. Chuaqui RF, Englert CR, Strup SE, Vocke CD, Zhuang Z, Duray PH, Bostwick DG, Linehan WM, Liotta LA and Emmert-Buck MR: Identification of a novel transcript up-regulated in a clinically aggressive prostate carcinoma. *Urology*, 50:302-307, 1997.

34. Thigpen AE, Cala KM, Guileyardo JM, Molberg KH, McConnell JD, and Russell DW: Increased expression of early growth response-1 messenger ribonucleic acid in prostate adenocarcinoma. *J. Urol.*, 155:975-981, 1996.

35. Wang FL, Wang Y, Wong WK, Liu Y, Addivinola FJ, Liang P, Chen LB, Kantoff PW and Pardee AB: Two differentially expressed genes in normal human prostate tissues and in carcinoma. *Cancer Res.*, 56:3634-3637, 1996.

36. Schleicher RL, Hunter SB, Zhang M, Zheng M, Tan W, Bandea CI, Fallon MT, Bostwick DG, and Varma VA: Neurofilament heavy chain-like messenger RNA and protein are present in benign prostate and down regulated in prostate carcinoma. *Cancer Res.*, 57:3532-3536, 1997.

37. O'Keefe, DS, Su, SL, Bacich DJ, Horiguchi Y, Luo Y, Powell CT, Zandvliet D, Russell PJ, Molloy PL, Nowak, NJ, Shows, TB, Mullins, C, Vonder Haar RA, Fair WR, and Heston WD: Mapping, genomic organization and promoter analysis of the human prostate-specific membrane antigen gene. *Biochim Biophys Acta*, 1443(1-2):113-127, 1998.

38. Silver DA, Pellicer I, Fair WR, Heston, WD, and Cordon-Cardo C: Prostate-specific membrane antigen expression in normal and malignant human tissues. *Clin Cancer Res*, 3(1):81-85, 1997.

39. He WW, Sciavolino PJ, Wing J, Augustus M, Hudson P, Meissner PS, Curtis RT, Shell BK, Bostwick DG, Tindall DJ, Gelmann EP, Abate-Shen C, and Carter KC: A novel prostate-specific, androgen-regulated homeobox gene (NKX3.1) that maps to 8p21, a region frequently deleted in prostate cancer. Genomics 43(1):69-77, 1997.

40. Crespi MD, Jurkevitch E, Poiret M, d'Aubenton-Carafa Y, Petrovics G, Kondorosi E, and Kondorosi A: Enod 40, a gene expressed during nodule organogenesis, codes for a non-translatable RNA involved in plant growth. *The EMBO J* 13:5099-5112, 1994.

41. Velleca MA, Wallace MC and Merlie JP: A novel synapse-associated non-coding RNA. *Mol. Cell Bio.* 14:7095-7104, 1994.

42. Takeda K. Ichijoh, Fujii M, Mochida Y, Saitoh M, Nishitoh H, Sampath TK and Miyazonok: Identification of a novel bone morphogenetic protein responsive gene that may function as non-coding RNA. *J. Biol. Chem.* 273:17079-17085, 1998.

43. Van de Sande K, Pawlowski K, Czaja I, Wieneke U, et al: Modification of phytohormone response by a peptide encode by ENOD 40 of legumes and a non-legume. *Science* 273:370-373.

44. Hao Y, Crenshaw T, Moulton T, Newcomb E and Tycko B: Tumor suppressor activity of H19RNA. *Nature.* 365:764-767, 1993.

45. Neumaier M, Gerhard M, Wagener C: Diagnosis of micrometastases by the amplification of tissue specific genes. *Gene.* 159(1) :43-47, 1995.

46. Gaddipati J, McLeod D, Sesterhenn I, Hussussian C, Tong Y, Seth P, Dracopoli N, Moul J and Srivastava S: Mutations of the p16 gene product are rare in prostate cancer. *The Prostate.* 30:188-194, 1997.

47. Davis LD, Sesterhenn IA, Moul JW and Srivastava S: Characterization of prostate cancer cells immortalized with E6/E7 genes. Int. Symp. On Biol. Of Prost. Growth Proceedings, National Institutes of Health., 77, 1998.

48. Wilkinson, D., & Green, J. (1990) in *Post implantation Mammalian Embryos*, eds. Copp, A.J. & Cokroft, D.L. (Oxford University Press, London), pp. 155-171.

49. Erdmann, V.A., Szymanski, M., Hochberg, A., de Groot, N., & Barciszewski, J. (1999) *Nucleic Acids Research* 27, 192-195.

50. Askew, D.S., & Xu, F. (1999) *Histol Histopatho.* 14, 235-241.

51. Bussemakers, M.J.H., Van Bokhoven, A., Verhaegh, G.W., Smit, F.P., Karthaus, H.F., Schalken, J.A., Debruyne, F.M., Ru, N., & Isaacs, W.B. (1999) *Cancer Res.* 59, 5975-5979.

52. Lanz, R.B., McKenna, N.J., Onate, S.A., Albrecht, U., Wong, J., Tsai, S.Y., Tsai, M.J., & O'Mally, B.W. (1999) *Cell* 97, 17-27.

53. Srikantan V, Zou Z, Petrovics G, Xu L, Augustus M, Davis L, Livezey JR, Connell T, Sesterhenn IA, Yoshino K, Buzard GS, Mostofi FK, McLeod DG, Moul JW, and Srivastava S: PCGEM1: A Novel Prostate Specific Gene is Overexpressed in Prostate Cancer. Submitted to *Proceedings of the National Academy of Sciences*.

We claim:

1. An isolated nucleic acid molecule selected from:

(a) the polynucleotide sequence of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:8;

(b) an isolated nucleic acid molecule that hybridizes to either strand of a denatured, double-stranded DNA comprising the nucleic acid sequence of (a) under conditions of moderate stringency in about 50% formamide and about 6X SSC at about 42°C with washing conditions of approximately 60°C, about 0.5X SSC, and about 0.1% SDS;

(c) an isolated nucleic acid molecule that hybridizes to either strand of a denatured, double-stranded DNA comprising the nucleic acid sequence of (a) under conditions of high stringency in about 50% formamide and about 6X SSC, with washing conditions of approximately 68°C, about 0.2X SSC, and about 0.1% SDS;

(d) an isolated nucleic acid molecule derived by *in vitro* mutagenesis from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:8;

(e) an isolated nucleic acid molecule degenerate from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:8, as a result of the genetic code; and

(f) an isolated nucleic acid molecule selected from the group consisting of human PCGEM1 DNA, an allelic variant of human PCGEM1 DNA, and a species homolog of PCGEM1 DNA.

2. A recombinant vector that directs the expression of the nucleic acid molecule of claim 1.

3. A host cell transfected or transduced with the vector of claim 2.

4. The host cell of claim 3 selected from bacterial cells, yeast cells, and animal cells.

5. An isolated nucleic acid molecule comprising the polynucleotide sequence selected from SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, and SEQ ID NO: 22.

6. A method of detecting prostate cancer in a patient, the method comprising:

(a) detecting PCGEM1 mRNA in a biological sample from the patient; and

(b) correlating the amount of PCGEM1 mRNA in the sample with the presence of prostate cancer in the patient.

7. The method according to claim 6, wherein step (a) includes:

(a) isolating RNA from the sample;

(b) amplifying a PCGEM1 cDNA molecule;

(c) incubating the PCGEM1 cDNA with the nucleic acid according to claim 1 or 5; and

(d) detecting hybridization between the PCGEM1 cDNA and the nucleic acid.

8. The method according to claim 7, wherein the PCGEM1 cDNA is amplified with at least two nucleotide sequences selected from SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, and SEQ ID NO: 22.

9. The method according to claim 8, wherein the at least two nucleotide sequences are SEQ ID NO:15 and SEQ ID NO:22.

10. A method according to claim 6, wherein the biological sample is selected from blood, urine, and prostate tissue.

11. The method according to claim 10, wherein the biological sample is blood.

12. A vector, comprising a PCGEM1 promoter sequence operatively linked to a nucleotide sequence encoding a cytotoxic protein.

13. The vector of claim 12, wherein the PCGEM1 promoter sequence is a nucleic acid molecule comprising the polynucleotide sequence of SEQ ID NO:3.

14. A method of selectively killing a prostate cancer cell, the method comprising:

(a) introducing the vector according to claim 12 to the prostate cancer cell under conditions sufficient to permit selective cell killing.

15. The method according to claim 14, wherein the cytotoxic protein is selected from ricin, abrin, diphtheria toxin, p53, thymidine kinase, tumor necrosis factor, cholera toxin, *Pseudomonas aeruginosa* exotoxin A, ribosomal inactivating proteins, and mycotoxins.

16. A method of identifying an androgen-responsive cell line, the method comprising:

(a) obtaining a cell line suspected of being androgen responsive,

(b) incubating the cell line with an androgen; and

(c) detecting PCGEM1 mRNA in the cell line,

wherein an increase in PCGEM1 mRNA, as compared to an untreated cell line, correlates with the cell line being androgen responsive.

17. A method of measuring the responsiveness of a prostate tissue to hormone-ablation therapy, the method comprising:

(a) treating the prostate tissue with hormone ablation therapy; and

(b) measuring PCGEM1 mRNA in the prostate tissue following hormone ablation therapy,

wherein a decrease in PCGEM1 mRNA, as compared to an untreated cell line, correlates with the prostate tissue responding to hormone ablation therapy.

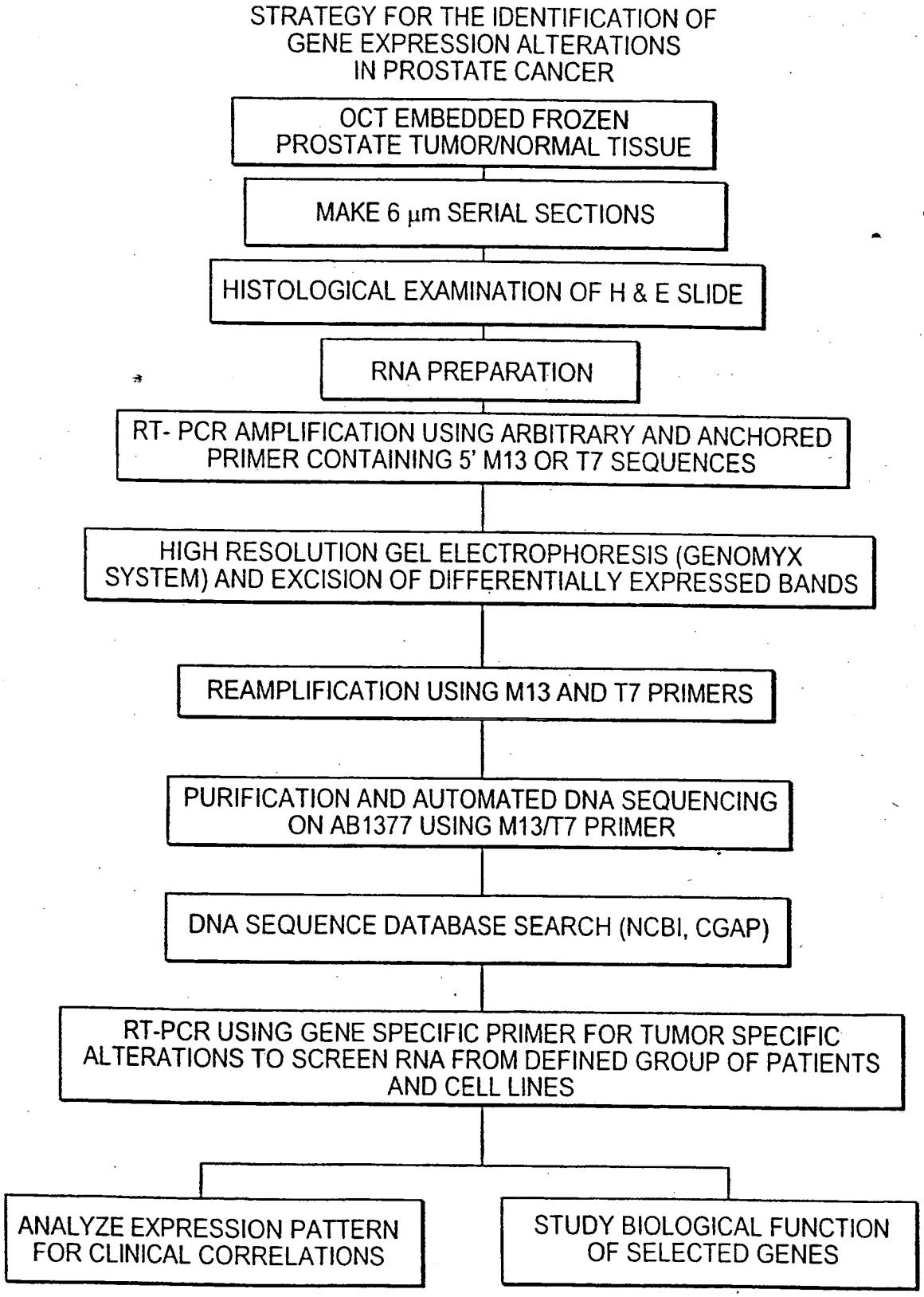
STRATEGY FOR THE IDENTIFICATION OF GENE EXPRESSION ALTERATIONS IN PROSTATE CANCER
OCT EMBEDDED FROZEN PROSTATE TUMOR/NORMAL TISSUE
MAKE 6 µm SERIAL SECTIONS
HISTOLOGICAL EXAMINATION OF H & E SLIDE
RNA PREPARATION
RT- PCR AMPLIFICATION USING ARBITRARY AND ANCHORED PRIMER CONTAINING 5' M13 OR T7 SEQUENCES
HIGH RESOLUTION GEL ELECTROPHORESIS (GENOMYX SYSTEM) AND EXCISION OF DIFFERENTIALLY EXPRESSED BANDS
REAMPLIFICATION USING M13 AND T7 PRIMERS
PURIFICATION AND AUTOMATED DNA SEQUENCING ON AB1377 USING M13/T7 PRIMER
DNA SEQUENCE DATABASE SEARCH (NCBI, CGAP)
RT-PCR USING GENE SPECIFIC PRIMER FOR TUMOR SPECIFIC ALTERATIONS TO SCREEN RNA FROM DEFINED GROUP OF PATIENTS AND CELL LINES
ANALYZE EXPRESSION PATTERN FOR CLINICAL CORRELATIONS
STUDY BIOLOGICAL FUNCTION OF SELECTED GENES

FIG. 1

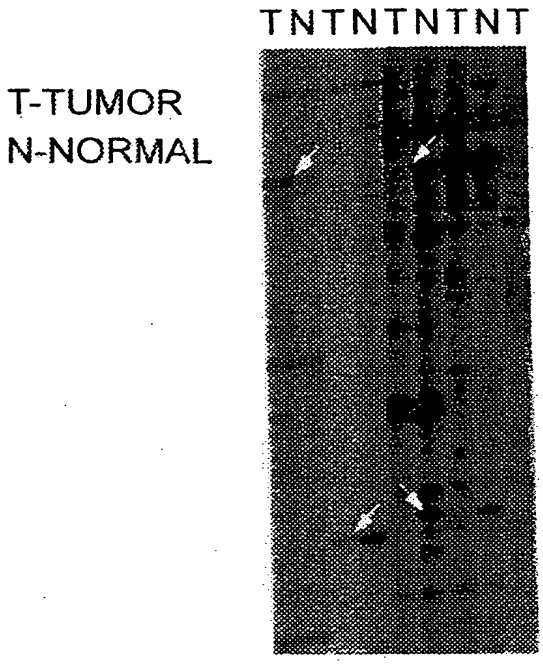

FIG. 2

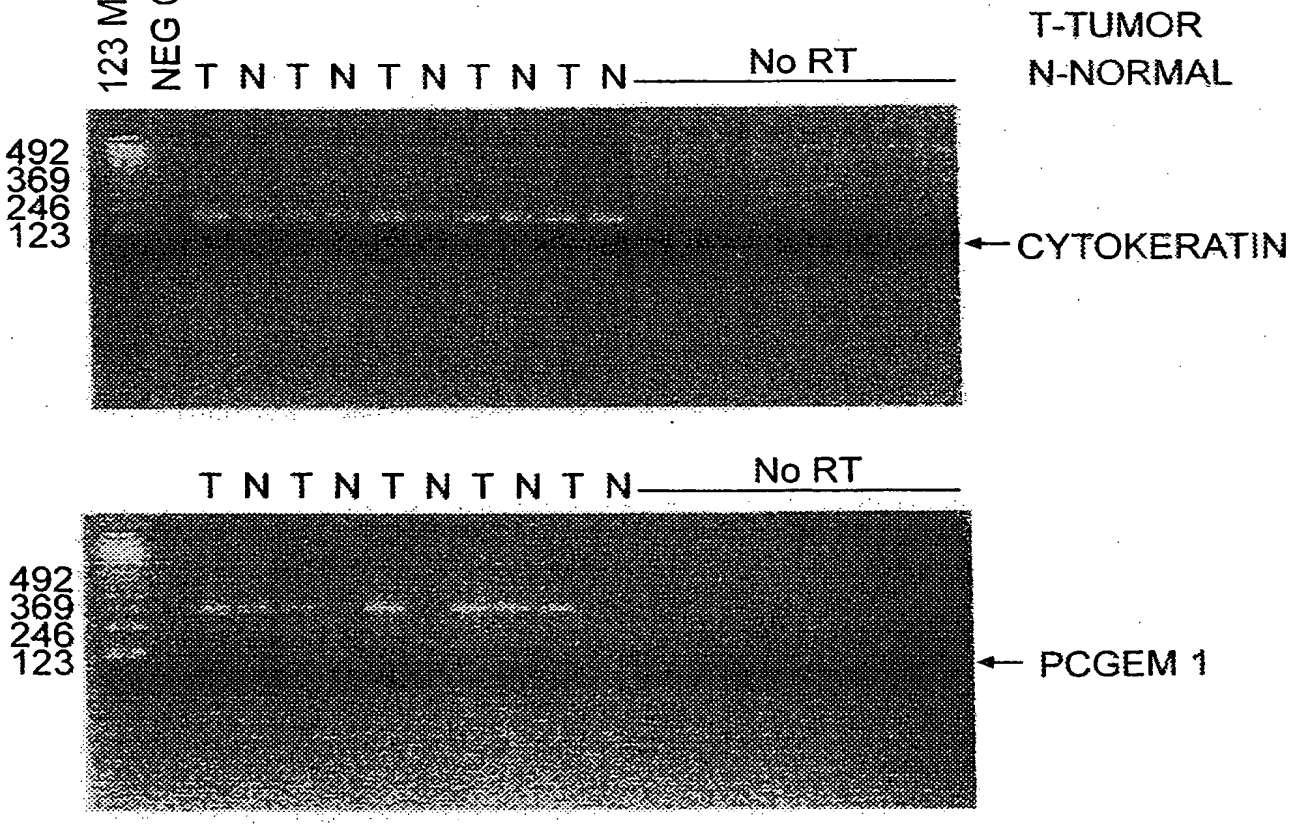

FIG. 3

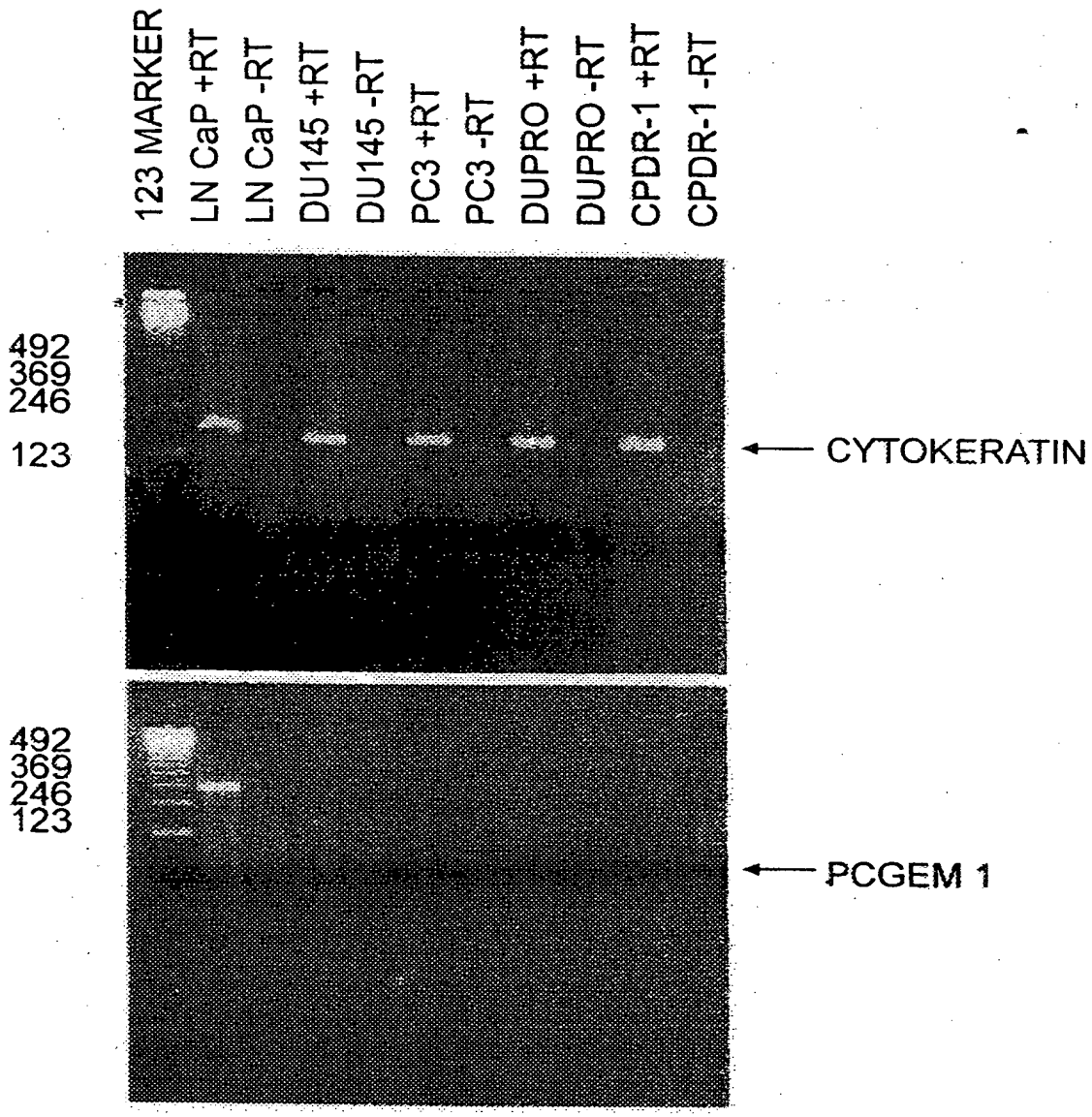

FIG. 4

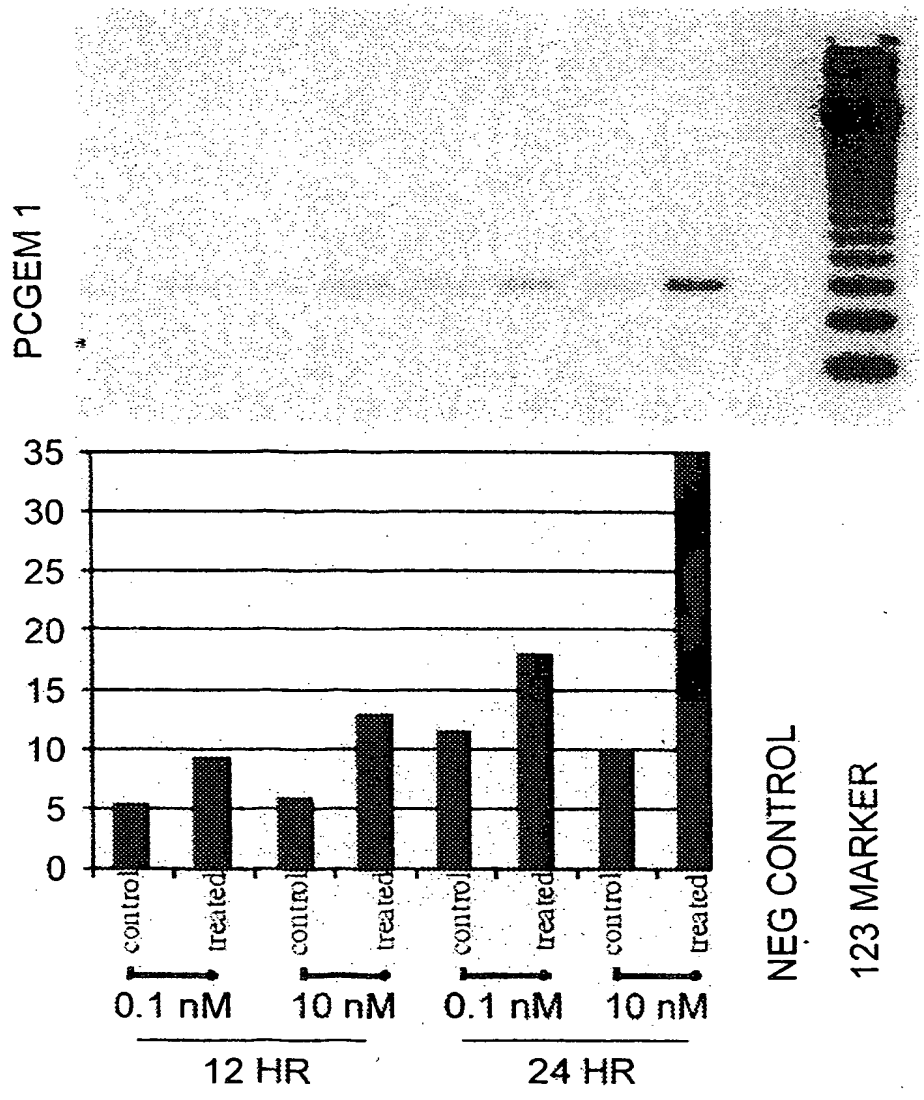

FIG. 5A

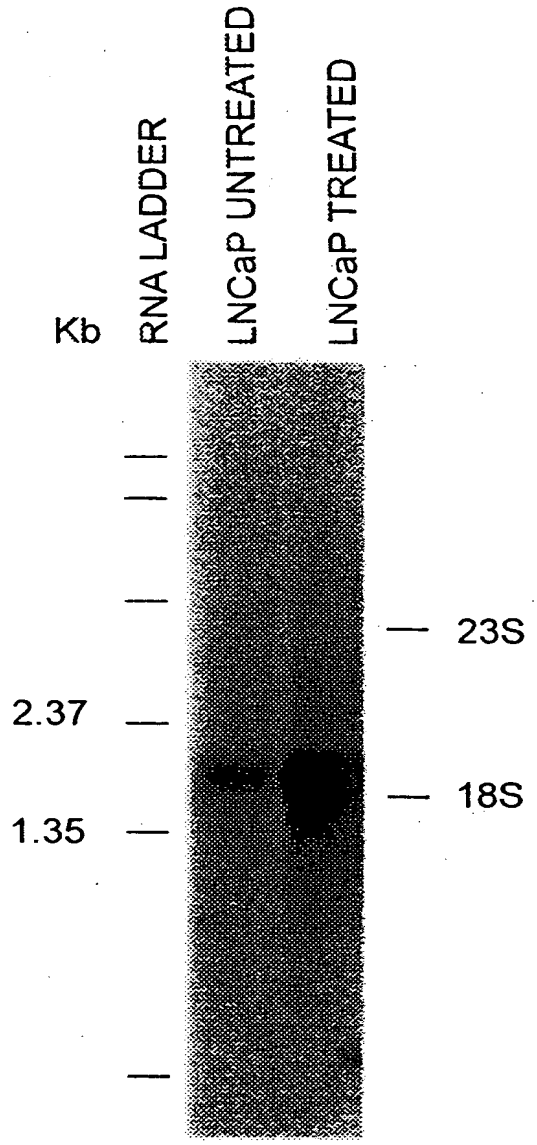

FIG. 5B

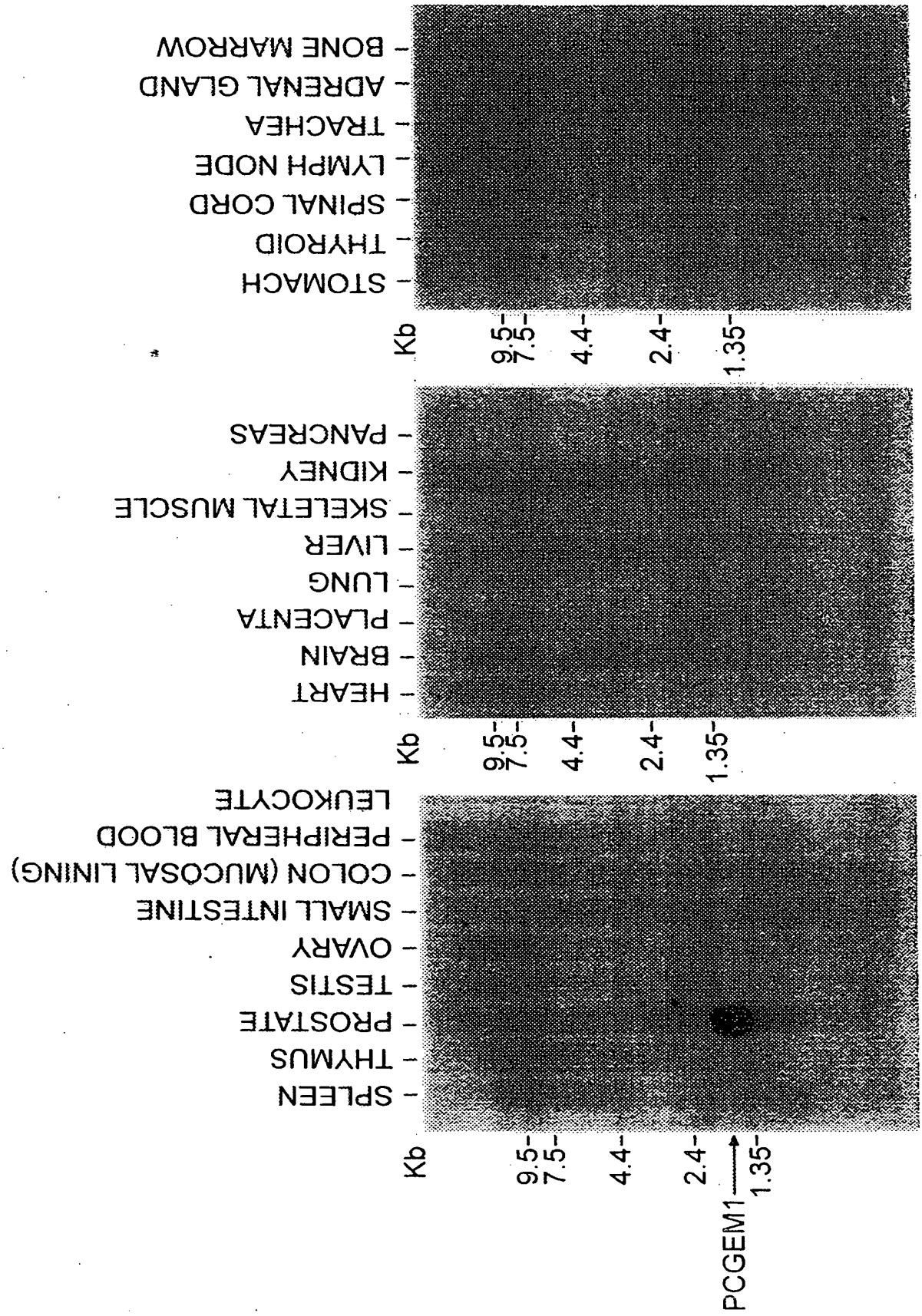

FIG. 6A

whole brain	amygdala	caudate nucleus	cere-bellum	cerebral cortex	frontal lobe	hippo-campus	medulla oblongata
occipital lobe	putamen	substantia nigra	temporal lobe	thalamus	nucleus accumbens	spinal cord	
heart	aorta	skeletal muscle	colon	bladder	uterus	prostate	stomach
testis	ovary	pancreas	pituitary gland	adrenal gland	thyroid gland	salivary gland	mammary gland
kidney	liver	small intestine	spleen	thymus	peripheral leukocyte	lymph node	bone marrow
appendix	lung	trachea	placenta				
fetal brain	fetal heart	fetal kidney	fetal liver	fetal spleen	fetal thymus	fetal lung	
yeast total RNA 100 ng	yeast tRNA 100 ng	*E. coli* rRNA 100 ng	*E. coli* DNA 100 ng	Poly r(A) 100 ng	human C_0t1 DNA 100 ng	human DNA 100 ng	human DNA 500 ng

FIG. 6B

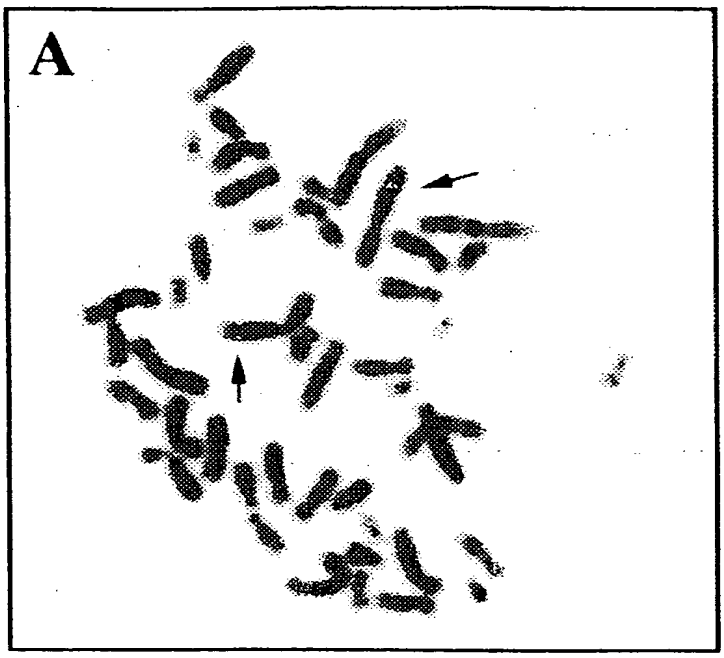

FIG. 7A

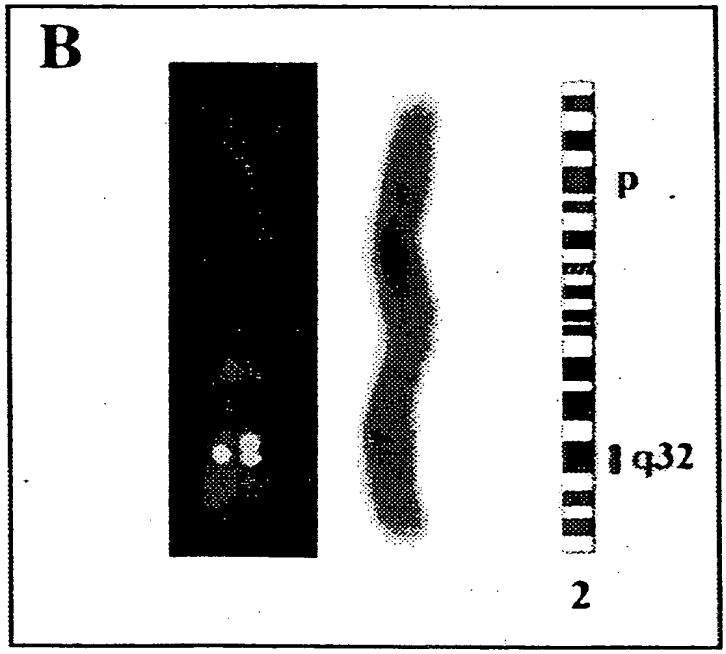

FIG. 7B

cDNA sequence of PCGEM1 Seq.ID No .1

```
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GGTGGAGGTT TTTAAAGAGC TATTTATAGG TCCCTGGACA GCATCTTTTT TCAATTAGGC AGCAACCTTT  140
TTGCCCTATG CCGTAACCTG TGTCTGCAAC TTCCTCTAAT TGGGAAATAG TTAAGCAGAT TCATAGAGCT  210
GAATGATAAA ATTGTACTAC GAGATGCACT GGGACTCAAC GTGACCTTAT CAAGTGAGCA GGCTTGGTGC  280
ATTTGACACT TCATGATATC ATCCAAAGTG GAACTAAAAA CAGCTCCTGG AAGAGGACTA TGACATCATC  350
AGGTTGGGAG TCTCCAGGGA CAGCGGACCC TTTGGAAAAG GACTAGAAAG TGTGAAATCT ATTAGTCTTC  420

GATATGAAAT TGTCTGTCTC TGTAAAAGCA TTTCATATTT ACAAGACACA GGCCTACTCC TAGGGCAGCA  490
AAAAGTGGCA ACAGGCAAGC AGAGGGAAAA GAGATCATGA GGCATTTCAG AGTGCACTGT CTTTTCATAT  560
ATTTCTCAAT GCCGTATGTT TGGTTTTATT TTGGCCAAGC ATAACAATCT GCTCAAGAAA AAAAAATCTG  630
GAGAAAACAA AGGTGCCTTT GCCAATGTTA TGTTTCTTTT TGACAAGCCC TGAGATTTCT GAGGGGAATT  700
CACATAAATG GGATCAGGTC ATTCATTTAC GTTGTGTGCA AATATGATTT AAAGATACAA CCTTTGCAGA  770
GAGCATGCTT TCCTAAGGGT AGGCACGTGG AGGACTAAGG GTAAAGCATT CTTCAAGATC AGTTAATCAA  840

GAAAGGTGCT CTTTGCATTC TGAAATGCCC TTGTTGCAAA TATTGGTTAT ATTGATTAAA TTTACACTTA  910
ATGGAAACAA CCTTTAACTT ACAGATGAAC AAACCCACAA AAGCAAAAAA TCAAAAGCCC TACCTATGAT  980
TTCATATTTT CTGTGTAACT GGATTAAAGG ATTCCTGCTT GCTTTTGGGC ATAAATGATA ATGGAATATT 1050
TCCAGGTATT GTTTAAAATG AGGGCCCATC TACAAATTCT TAGCAATACT TTGGATAATT CTAAAATTCA 1120
GCTGGACATT GTCTAATTGT TTTTTATATA CATCTTTGCT AGAATTTCAA ATTTTAAGTA TGTGAATTTA 1190
GTTAATTAGC TGTGCTGATC AATTCAAAAA CATTACTTTC CTAAATTTTA GACTATGAAG GTCATAAATT 1260

CAACAAATAT ATCTACACAT ACAATTATAG ATTGTTTTTC ATTATAATGT CTTCATCTTA ACAGAATTGT 1330
CTTTGTGATT GTTTTTAGAA AACTGAGAGT TTTAATTCAT AATTACTTGA TCAAAAAATT GTGGGAACAA 1400
TCCAGCATTA ATTGTATGTG ATTGTTTTTA TGTACATAAG GAGTCTTAAG CTTGGTGCCT TGAAGTCTTT 1470
TGTACTTAGT CCCATGTTTA AAATTACTAC TTTATATCTA AAGCATTTAT GTTTTTCAAT TCAATTTACA 1540
TGATGCTAAT TATGGCAATT ATAACAAATA TTAAAGATTT CGAAATAGAA AAAAAAAAAA AAA          1603
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FIG. 8

cDNA sequence of PCGEM1 Seq. ID No .2

```
GCGGCCGCGT CGACGCAACT TCCTCTAATT GGGAAATAGT TAAGCAGATT CATAGAGCTG AATGATAAAA   70
TTGTACTTCG AGATGCACTG GGACTCAACG TGACCTTATC AAGTGAGATG GAGTCTTGCC CTGTCTCCAA  140
GGCTGGAGCC CAATGGTGTG ATCTTGGCTC ACTGCAACCT CCACCTCCCA GGTTCAAACG TTTCTCCTGC  210
CTCAGCCTCC CAAGTAACTG GGATTACAGC AGGCTTGGTG CATTTGACAC TTCATGATAT CAGCCAAAGT  280
GGAACTAAAA ACAGCTCCTG GAAGAGGACT ATGACATCAT CAGGTTGGGA GTCTCCAGGG ACAGCGGACC  350
CTTTGGAAAA GGACTAGAAA GTGTGAAATC TATTAGTCTT CGATATGAAA TTCTCTGTCT CCGTAAAAGC  420
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ATGTTTCTTT TTGACAAGCC CTGAGATTTC TGAGGGGAAT TCACATAAAT GGGATCAGGT CATTCATTTA  700
CGTTGTGTGC AAATATGATT TAAAGATACA ACCTTTGCAG AGAGCATGCT TTCCTAAGGG TAGGCACGTG  770
GAGGACTAAG GGTAAAGCAT TCTTCAAGAT CAGTTAATCA AGAAAGGTGC TCTTTGCATT CTGAAATGCC  840
CTTGTTGCAA ATATTGGTTA TATTGATTAA ATTTACACTT AATGGAAACA ACCTTTAACT TACAGATGAA  910
CAAACCCCAC AAAAGCAAAA AATCAAAAGC CCTACCTATG ATTTCATATT TTCTGTGTAA CTGGATTAAA  980
GGATTCCTGC TTGCTTTTGG GCATAAATGA TAATGGAATA TTTCCAGGTA TTGTTTAAAA TGAGGGCCCA 1050
TCTACAAATT CTTAGCAATA CTTTGGATAA TTCTAAAATT CAGCTGGACA TTGTCTAATT GTTTTTTATA 1120
TACATCTTTG CTAGAATTTC AAATTTTAAG TATGTGAATT TAGTTAATTA GCTGTGCTGA TCAATTCAAA 1190
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GTTTTAATTC ATAATTACTT GATCAAAAAA TTGTGGGAAC AATCCAGCAT TAATTGTATG TGATTGTTTT 1400
TATGTACATA AGGAGTCTTA AGCTTGGTGC CTTGAAGTCT TTTGTACTTA GTCCCATGTT TAAAATTACT 1470
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FIG. 9

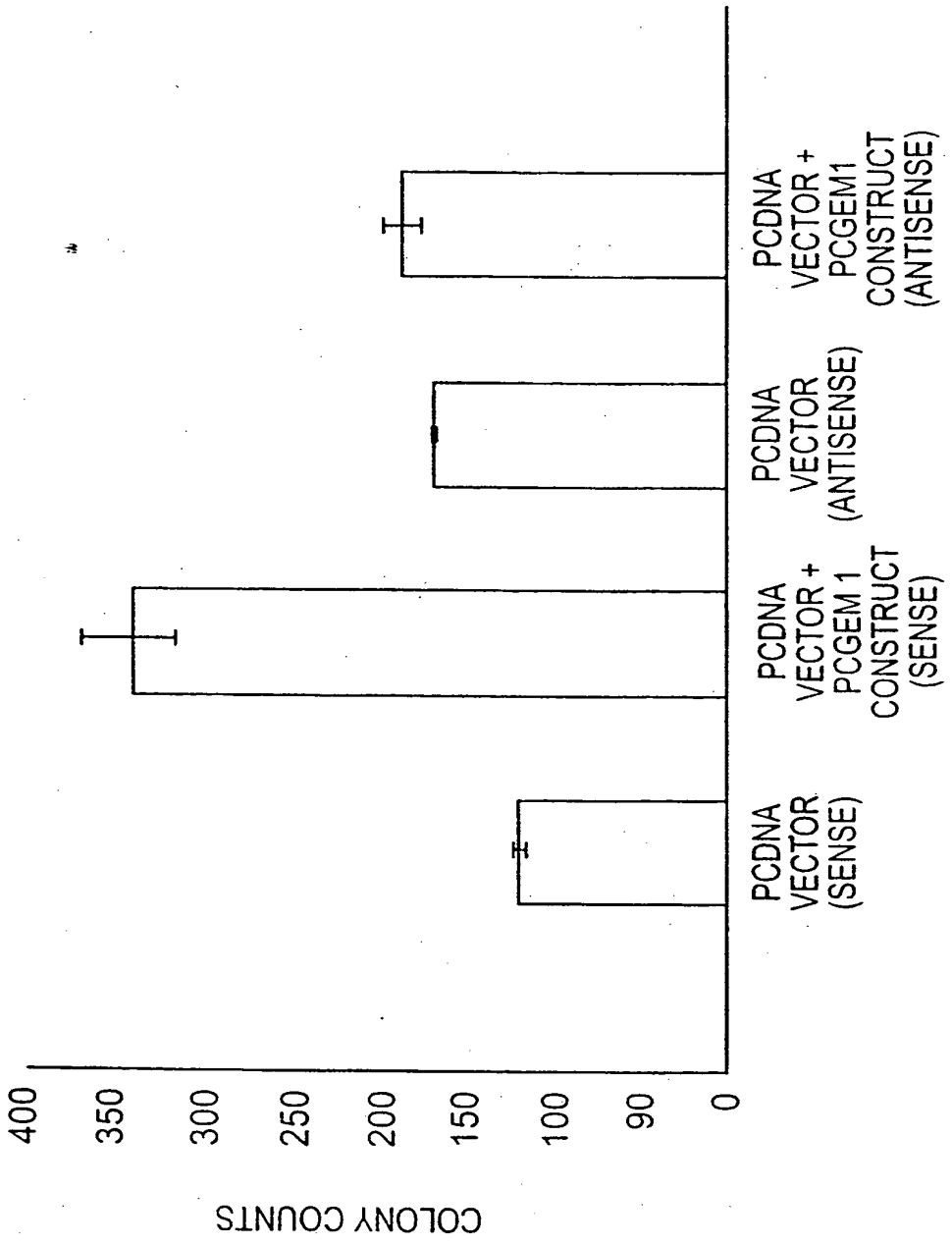
COLONY COUNTS
400
350
300
250
200
150
100
90
0
PCDNA VECTOR (SENSE)
PCDNA VECTOR + PCGEM 1 CONSTRUCT (SENSE)
PCDNA VECTOR (ANTISENSE)
PCDNA VECTOR + PCGEM1 CONSTRUCT (ANTISENSE)

FIG. 10

cDNA sequence of PCGEM1 Promoter Region Seq.ID No.3

TCCCTCTTGC GTTCTGCAAT TTCTGAAAAA AAGATGTTTA TTGCAAAGTG ATATGAGCAC TGGAAAGGTA 70
CTAATTCCAA TTTGATTCTA ATTGGATGAG TGACATGGGT AAGCGATTCT AAGCATTTGT GTTTTTTTTA 140
GTAGTATGGA ATTTAATTAG TTCTCAGTAT GTTAGTGAAG ATGAATGAAA ACATGCATAT GTTTCCATGT 210
ATTATAAAATA TTTTAAAATG CAAAAAATTA TTCTAATGAA TATATAAATA TAAAGCATAA CAATAATAAT 280
ACAATACCAC CCATAAAGTC ATCATCTAAT TTAAAAACTA AAACATTAAC ACTTGAATCT CCCCCATTGC 350
AACATCTTTC CCGACTTGTG TGTTTTTTTC TTTTGCTTTT AAAATTTTTG TTTTATCATA TGTCTGCATA 420
AGATTATATA GCTTTCCTTG TTTTAAGCTT TTTAAATAAT ATATTGTAGT TATATTATTT GTGCTTTGCT 490
TTTTTTACTT AACATTATGG TTCTAAAATT CAGTAATGTG TTGGGCATGT ATAATTTGTT TATTTTTAAT 560
CTCTTTGACA TTCGACTATA TAAATTTCAG TTTGTTTATT GACTCCTTTG TCTATAGATA CTCTGCTATT 630
TCTGTTTTTG CTGTTACAAA AATAATGCTG TTTTAAATTT CATTTTGTAT ACTTTTTTGA GGCATGTGTA 700
TGAGTTATTC TAAGGTAAAA AAATAAGAAA AAATTGCTGG GTTATAAGAT TGTCACATGC TCGAATTTAC 770
AAGATAATGC CAAATCATTT TTCAAAGTAA TTATACCTAT TTATACTACC GGTATGAGTA TATTGGTGCC 840
CACATAGTTG CTTGTTCTGC CAAAGTTTGG TATGATCGAA CAATAATTTT TGCCCATCAA ATGGCATAAA 910
ATAAAATCTC AGTGTGCTTT TAATTTGCAT TTTCTATGTT TAAGAATTGT TTCTTTTTTA ACCATTTATA 980
ATTTACTTTT GCTGAAATGC TTGCTTATTA TTTTTGCTCC CCATTTTTTC CTATTGGATT GCTTTTCTCA 1050
TTAATTTATA AGAATTTTAT ATGGTTTAGA TACTAATTAT TATATTACTG AAAATACCTT TATCAGTTTG 1120
TTGTGTACTT TCTACTTTAT GTCTTGTGAT GGATAAAAGT TTTAAATTGT ATTGTGTTGA AGTTAACATT 1190
TTTAAATTTT ATAATCAGCA TCTTTAATAA TCTCTTTMTA AAATTTTCCT TTACATAGAT GTCATAAAGA 1260
TACATCTCTA TAATTTCTTA TTTTTTTGGC ATATGTTCAT TAAGTCATTT TATCATTTTT TAGTAATAAA 1330
TTGCAGTTAT TTATGAAACA AATAATTTTT AAAATTATAT ATGCTTTCTT TAAAAATTGA TCTTAGCATG 1400
CTTCACTATG AAGCTTGAGG CTTCACTGCA CGTTGTACTG AAATTATGTA TAAAACAGTG GTTCTGAAAA 1470
TCTCTGAGTT CATGACACCT TTAGTGTCTC AGGTTTTTTT GCTTTTGTTC TTGTTTTTTC TCACAAAGCA 1540
CCTAAGTTAA ATAAAAACAA AGCACAAAGC TATCAGCTTC ATGTATTAAG TAGTAAGCTC CCATGTTAAC 1610
AGTTGTAACT TGCCTGGTGC CCAATAGATG TCACTCTGTT TTCCTAGAAA CTTTAAAATA TCCCTCAGTG 1680
CTCCTGTTAA TTCATGGTAG TGCCCCAAGG CACTCTGGCA CCCAGTTTTG GAACTGCAGT TTTAAAAGTC 1750
ATAAATTGAA TGAAAATGAT AGCAAAGGTG GAGGTTTTTA AAGAGCTATT TATAGGTCCC TGGACAGCA 1819

FIG. 11

cDNA sequence of PCGEM1 PROBE Seq.ID No.4

TTTTTTCAAT TAGGCAGCAA CCTTTTTGCC CTATGCCGTA ACCTGTGTCT GCAACTTCCT CTAATTGGGA 70
AATAGTTAAG CAGATTCATA GAGCTGAATG ATAAAATTGT ACTACGAGAT GCACTGGGAC TCAACGTGAC 140
CTTATCAAGT GAGCAGGCTT GGTGCATTTG ACACTTCATG ATATCATCCA AAGTGGAACT AAAAACAGCT 210
CCTGGAAGAG GACTATGACA TCATCAGGTT GGGAGTCTCC AGGGACAGCG GACCCTTTGG AAAAGGACTA 280
GAAAGTGTGA AATCTATTAG TCTTCGATAT GAAATTCTCT GTCTCTGTAA AAGCATTTCA TATTTACAAG 350
ACACAGGCCT ACTCCTAGGG CAGCAAAAAG TGGCAACAGG CAAGCAGAGG GAAAAGAGAT CATGAGGCAT 420
TTCAGAGTGC ACTGTCTTTT CATATATTTC TCAATGCCGT ATGTTTGGTT TTATTTTGGC CAAGCATAAC 490
AATCTGCTCA AGAAAAAAAA ATCTGGAGAA AACAAAGGTG CCTTTGCCAA TGTTATGTTT CTTTTTGACA 560
AGCCCTGAGA TTTCTGAGGG GAATTCACAT AAATGGGATC AGGTCATTCA TTTACGTTGT GTGCAAATAT 630
GATTTAAAGA TACAACCTTT GCAGAGAGCA TGCTTTCCTA AGGGTAGGCA CGTGGAGGAC TAAGGGTAAA 700
GCATTCTTCA AGATCAGTTA ATCAAGAAAG GTGCTCTTTG CATTCTGAAA TGCCCTTGTT GCAAATATTG 770
GTTATATTGA TTAAATTTAC ACTTAATGGA AACAACCTTT AACTTACAGA TGAACAAACC CACAAAAGCA 840
AAAAATCAAA AGCCCTACCT ATGATTTCAT ATTTTCTGTG TAACTGGATT AAAGGATTCC TGCTTGCTTT 910
TGGGCATAAA TGATAATGGA ATATTTCCAG GTATTGTTTA AAATGAGGGC CCATCTACAA ATTCTTAGCA 980
ATACTTTGGA TAATTCTAAA ATTCAGCTGG ACATTGTCTA ATTGT 1025

FIG. 12

PCGEM1 Primers Used for PCR

PCR PRIMER 1 (SEQ ID No.5)

Sense Primer 5' TGCCTCAGCCTCCCAAGTAAC 3'

PCR PRIMER 2 (SEQ ID No.6)

Antisense Primers 5' GGCCAAAATAAAACCAAACAT 3'

PCR PRIMER 3 (SEQ ID No.7)

Sense Primer 5' TGGCAACAGGCAAGCAGAG 3'

FIG. 13

Complete Genomic DNA sequence of PCGEM1 gene.

TCCCTCTTGCGTTCTGCAATTTCTGAAAAAAAGATGTTTATTGCAAAGTGATATGAGCACTGGAAAGGTACTAATTCCAA
TTTGATTCTAATTGGATGAGTGACATGGGTAAGCGATTCTAAGCATTTGTGTTTTTTTTAGTAGTATGGAATTTAATTAG
TTCTCAGTATGTTAGTGAAGATGAATGAAAACATGCATATGTTTCCATGTATTATAAATATTTTAAAATGCAAAAAATTA
TTCTAATGAATATATAAATATAAAGCATAACAATAATAATACAATACCACCCATAAAGTCATCATCTAATTTAAAAACTA
AAACATTAACACTTGAATCTCCCCCATTGCAACATCTTTCCCGACTTGTGTGTTTTTTTCTTTTGCTTTTAAAATTTTTG
TTTTATCATATGTCTGCATAAGATTATATAGCTTTCCTTGTTTTAAGCTTTTTAAATAATATATTGTAGTTATATTATTT
GTGCTTTGCTTTTTTTACTTAACATTATGGTTCTAAAATTCAGTAATGTGTTGGGCATGTATAATTTGTTTATTTTTAAT
CTCTTTGACATTCGACTATATAAATTTCAGTTTGTTTATTGACTCCTTTGTCTATACATACTCTGCTATTTCTGTTTTTG
CTGTTACAAAAATAATGCTGTTTTAAATTTCATTTTGTATACTTTTTTGAGGCATGTGTATGAGTTATTCTAAGGTAAAA
AAATAAGAAAAAATTGCTGGGTTATAAGATTGTCACATGCTCGAATTTACAAGATAATGCCAAATCATTTTTCAAAGTAA
TTATACCTATTTATACTACCGGTATGAGTATATTGGTGCCCACATAGTTGCTTGTTCTGCCAAAGTTTGGTATGATCGAA
CAATAATTTTTGCCCATCAAATGGCATAAAATAAAATCTCAGTGTGCTTTTAATTTGCATTTTCTATGTTTAAGAATTGT
TTCTTTTTTAACCATTTATAATTTACTTTTGCTGAAATGCTTGCTTATTATTTTTGCTCCCCATTTTTTCCTATTGGATT
GCTTTTCTCATTAATTTATAAGAATTTTATATGGTTTAGATACTAATTATTATATTACTGAAAATACCTTTATCAGTTTG
TTGTGTACTTTCTACTTTATGTCTTGTGATGGATAAAAGTTTTAAATTGTATTGTCTTGAAGTTAACATTTTTAAATTTT
ATAATCAGCATCTTTAATAATCTCTTTATAAAATTTTCCTTTACATAGATGTCATAAAGATACATCTCTATAATTTCTTA
TTTTTTTGGCATATGTTCATTAAGTCATTTTATCATTTTTTAGTAATAAATTGCAGTTATTTATGAAACAAATAATTTTT
AAAATTATATATGCTTTCTTTAAAAATTGATCTTAGCATGCTTCACTATGAAGCTTGAGGCTTCACTGCACGTTGTACTG
TTGTTTTTTGTCACAAAGCACCTAAGTTAAATAAAAACAAAGCACAAAGCTATCAGCTTCATGTATTAAGTAGTAAGCTC
CCATGTTAACAGTTGTAACTTGCCTGGTGCCCAATAGATGTCACTCTGTTTTCCTAGAAACTTTAAAATATCCCTCAGTG
CTCCTGTTAATTCATGGTAGTGCCCCAAGGCACTCTGGCACCCAGTTTTGGAACTGCAGTTTTAAAAGTCATAAATTGAA
TGAAAATGATAGCAAAGGTGGAGGTTTTTAAAGAGCTATTTATACCTCCCTGGACAGCATCTTTTTTCAATTAGGCAGCA
ACCTTTTTGCCTATGCCGTAACTGTGTCTGCACTTCCTCTAATTGGGGTGAGTAAGAGATTTTGTTATGTATATAATAGC
TAAGAATATAGTAATAATCCCTTAAATCATGGTTATTTTTAAACTACTAACATTTAGAAGACAAAATAAAAATGCTTTGA
AAAGTATAGAGGTTTTAGTGTAATTAGCAGGGAATAATGAAATGATTTGATAGGGCTACTCAGTTTTGTATAACTTTGGT
GCTTTAAGTCTGAATGCAGAGCATGGATGTTGTGATCCAGCCTTTATATGTTTTCCCTGAAGAAGATTTAATTTATTTGG
CCTTTTGAGAAACACATTTGGCATTGTAATATGTTTTGCTTCCAGGTTCTATCTCCAAGGATAATTTGACAAAATCACAC
ATAAATTTATTTTCAGGGCACACAGTTTCCCTTTTAGGGAACTCACAGAGGTAGAGAGTAATACAATAATCACATTTGAA
TATTCAGTAAGTGAGGTCCTCATAGATCTTATGTGTATGTCACCATGTATATAATTTTGTTAATCACTAGATGTATGAGA
CAAGAAATTTGAGGAATCTTAACTAGAGATTAAAATCAGGGATTTAAATCAAAGAAACATTTAAATGCCTCCTTTATTAT
TTAAATACCTGCATGGGAGAATCATTGAAAAAAAAATAAAAAGCATACAACTTGGGAATATTATAAACCAAGAAGAATTT
GTTATTCTGGTTGATTTTTTTTTCAGGCTCCGCACAGGCAACTTACCTTTATCTCTTTGTGATTTTTATTTCTTGTTAAA
ATATACAGAAATAGTTAAGCAGATTCATAGAGCTGAATATAAAATTTACTACGAGATGCACTGGGACTCAACGTGACCTT
ATCAAGTGACTTATCAGTGAGGTGAGCATTCTTAATTCAGATAATGGAACTTATTATCATAATCTTTTGCTTATGCTATT
GTTGAGCTTAACTACTTATTCATATTTGCATATGCATATTGAGATAATATCATTTCATTAATTTCAGTACTGAACACTAA
TCTCCTAAGAGTAATTGTGAAAGTTTCAGATTGCACTATTTTTAACTATATATCTGTATGTTATCTTCATATATGCTTGA
ATAACTTATAAGCAATTGAAACTTTCAATTACAGTATACTATTGAAGCAAATCAACAAATATATACACATATCCATTAGC
AATAGTAGATAATTTTTGTAAATGTCCAGCACAGTTCTTCATATGTAGAGGATGTTCAAATTGGCTAAGTTCCTTTTCTC
TCTTAATTATTAGTATTTTTCCTACTGCTCTTTGTATAATTATTCCTTCCTCTTTAGCTCCAATCCTTACAATCTATTCT

FIG. 14

TAACATAGCAACTGGGAAGAAAGTTTTTAAACATAAACCAGATGATGTCACTCCACCCCACAAAACTTCCACTATTCTCT
GTCACACATAGAAAGAAAGAAAAAAAATATTGAAAACCTACAAAGACTTGCTATGATCTGGTCCAGGCTCTCCCTAAAAT
TTCATGTAATTTCCAGCCACTAGGCCTTTCTGGCTCTCCTTCAATCTCATTAGCCTTTTCACTACTACAAGTTAGACTGG
GTTTTGGCCGAGGTATTTCTTTTTTTCATATTTTGCCTTTGCCTAGATTGCTCTTCCAATAGATATTCACAATTGCATCA
TCATTTCTATATACGTGCTAAAAGGTTTCCTTGTCCAAAATAGCTTCAGTGACCACCTGATCTAGAATAGTCTCGATCAA
AAGTTTCTTTTCCTTTTCCTCACCACTTGATATTTATATCAAACATTTATTTGTGTAATTTATGTGTTTGTTTGTTTTCT
GTACTAGCATTATGATGACCATACTATTTGATGCCCCCCAAAAAATACTTTCGAGAATGACAGGGCAAAGCTAAAATAAT
TAAATTATATAATTTTGACATAGGCACTATTGACAAAAAGCAATTGATGTTATGATAGTGTTAGATCTATGAAATAGTAC
TATTTAAAAGTAATTCTCTGAAATACAATTTTCTAAAACTAAAAGCAGCATATGTACATGAAACACCAAAAAACTTCCTT
ATATTTATCACTGGAAGATTTAAAATAGTATAAGTAGTAACTTATTTAATATATTTTTGATTATTTAATTAATTTTATAG
TATCCAACTCTAATATAATGCCACTGGTATTTGTTCAAAATATTTTAATGTTGTCTATTTATTTTTAATTTGCCTAAAAA
TTATCTTAAATGAAAATTTTTGGTTAATAAATTTGAAAATACTGAAACCCTCATCTCCAGTCTCTGTGGATCCTAAAGTT
TTTAGTTGAGAAAATAATTTTTCTCTAGAGAATGAAGTAGCTTGTAAGCTTGGAGAAATTTCTGCTAAATAAATGATATT
ATCAACTCTTATTTTCTTCAATACGAAATATATAAATATTTCAGCTCATATATTTTTGCAGGTGCTATGCTTTTGCTTCC
AATCATAATTTCTGACAAATATTTTGGAAGTCAAAACTTGTCTTCTATTTTGTTATTTAAAATTATATAGACTACTTTTG
TAAACCTTTATACTATCAAATCATAGGCAATTTCAGTTTGATTTCATTCTGGTGCAGAATATAAGTTTATCCAAGTAAAA
CAGGAGTCACTTCAAAAGATTCCTCCCACTGACTGAGATATTCCAAAGCCAACTTTGCAAAATTTCAGAATTAAATATTA
TACTTCTTTGTACCTTCATTTTATTTGTTCAATTTTTCTTTGTGTTTGTAGAAAATTTTAATATTTTTCTGTTTTCAAGT
TTTGATTTTAATTTACTACTTTATAATTTTTAAAGGTAAGTTTTGTGAGGCTATATTCATTATGTGTTTTGAATAAAGAC
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AAATGCAGTACCAGTAGACTACATTTAGGCTGCTTAAAGTTAGTTCTTCTAAGTACCATATACTTTAAAATTTTAGCTAA
TGATGGAGAACAAAGACAGAAAGACTGTGTTACCATATTCTAGTTGGCCATTTTGTTTTGTTTTGAGAGACGTCACATCA
GCCTTATCATAAAAATTATTTGGTTTTACCATTTTGACTGTGAGCAAAATATACAGCATAATATACAAAATAAAATACAT
GTACATCTTCACAACTTCTTGTTTAGGATGCAATTATATATATATATATATATATTTATTATTATACTTTAAGTTCTA
GGGTACATGGCACCACGTGCAGGTTGTTACATATGTATACATGTGCCATGTTGGTGTGCTGCACCCATTAACTCGTCATT
TACATTAGGTGTATCTCCTAATGCTATCCCTCCCCTCTCTCCCCACCCCACAACAAGCCCCGGTGTGTGATGTTCCCCTT
CCTGTGTCCATGTGTTCTCATTGTTCAATTCCCACCTATGAGTGAGAACACGCAGTGTTTGCTTTTTTGTCCTTGCAATA
GTTTGCTGAGAATGATGGTTTCCAGCTTCATCCATGTCCCTACAAAGGACATGAACTCATCATTTTTTATGGCTGCATAG
TATTCCATGGTGTATATGTGCCACCATTTTCTTAATCCGAGTCTGTCCATTGTTGTTGGACATTTGGGTTGCAATTTTGA
GTTTCATGTGTAGCATGTATAGCACAACCAATTAAGATTTCTTTCTTTCTCTCTTTTTTTTTTTTTTTTGTTGAAATGGA
GTCTTGCCTGTCTCCAAGGCTGGAGCCCAATGGTGTGATCTTGGCTTACTGCAACCTCCACCTCCCGGGTTCAAGCGATT
CTCCTGCCTCAGCCATCCGAGTAGCTGGGACTATAGGCGTGCACCACCATGCCCAGCTAATTTTTGTATTTTTAGTACAG
ACGGGGTTTCACCACGGTGGCCAGGATGGTCTCAATTTCTTGACCTCATGATTCACCCGCCTTGGCCTCCCAAAGTGCTG
GGATTACAGGTGTGAACCACCAAGCCCGGCCTGTCACAAGTTTTTAGTGTTCTATTTTAATACAGAAATTAGATAAATCC
AAAGAGAAAGACATTTCATATGTGCGTAGAGTTGTCGGAAGAAATGAGAGTCTTATAAATAACTTTAAAAATTGTGAAGA
AATAAAGGCAAAATAGTCCTATGCAGTTTGATTTAAATATATTCTTAATAAGAGCTACTTTTGTGAAACCAGAATAATTG
AAACATGTAGATATGGATCTTCATTAGTGACTGACATAATATATTGTTATTGTTACTATTTTATTGTATCAGCCAACTAA
TATTGAGTGCTTTGTGTATCCTAAGCACTATGCTAAACACTGTACCAGTATTACCTGATATAATCATATTAATATTTATT

FIG. 14(cont'd-1)

ATTTCACTTTTCATATGAAAAAATTGAAGCACAGATTAAGACACTCCGAAATCATACCTCTATTGATTATCAGCACCAGG
ATTTGAATTGAGGCACTCTGATCCAGAGAAGCTTTTGTTTCCATGAAGGCTTATGTTGGGGAAAAATAATCAAATTGCCT
GTACCTCAGTTGTATAAATAAGAGGTTGGGTTGGTAGATGATTCTGGCTGATTCAGCAGAAAAGAAATTTATTCAAAGGA
TATCACACAGTTTTCATAACAGTTAAGAATACAGAGGAAACAGGGCACCAGGGCTAAGTACAGACCAAAGTCCAAAACCA
CTGCCAAAGTTGCAGCAAGGAGAACAGCACAAATTTGCTTGCTGTCACCCGCCACTAGATGCTTTTGTTTGGAGCCTTGA
ACTTGACTTACACTGCCACTGACATCAGCACCAGTGCTCTCTGTGTACTAGGAGGTGGAGTTGGTGACGTTGCTGAACTA
AAAGCAGATGTTTCTGCTGTGAAATAGATACCTAATACAGAACCTGATTCCTCATTCATTCCCTCCCCAAATCATATGCT
TGTAGTGTGGCTAGAGTTTCTGTTTCTCCTTGGTCCAGGCAGAATTTATGAAGCTTGCTATTTATCGCCTTAAAGATTAG
AAGAATATTCATAAGGTATTAGATTGCCATAAGGTTGAACAAATCAACATTCAACTTCAAGGATTCAACATTGTTTTGTT
TTCTTTTGGGATACCTCTGCAGCAGTTCAAATCTTATTTCTGCCCTTGGACAACCAGGTTTATAAATATTGCAGATTCTC
CACTGACTGCTTTGATCCTATCTTCTATATTTATGTATACTAATTAGCATATAATAAAAGATTATGTTACAGAATCTCAA
AATTAGTAATTATGAATTGAGATGGTGTTATACAGTACACTAACATCCAAGAGACTTGTTTATTCCAAGGAAAATATTTA
GAGATATTAAATGATATTTCTCATCCTTTAGACATATACATTTTTTAGCTTACAGCCTGCTTTAGGCAAGCAACAGACTC
TCAGGATCTGCTCCTACCAGGGTCTGAACATTTCCTCCCAGTTTTAAAGAAACAAATTCAAATAACATTGTAACCTCCAG
AGGAAAGTTCAAGGTCTTTTATAGTATTGTTTAAACAGTACAGCTGAGGAAACTAAAGACAGAGAAGTTAAATGCCTTGG
CACTTAGTCTAGATTTACAATAAACTCCTYTCTACTTAGGACCCACTAACAGGGGCTGCATTTACACCAAAACCATGAAG
GTGGCCCAAGTCATCACTGAGAAGTAGTACAAGCACCGAGGGAATGACTTCAACAGGAACAAGAAAGCGTGGAAGGAGAT
CCTAGCAGGAAGCTCCACAAGAAGATAGCATGTTACGTCTTGCATTGGATGAAGCAGGTTCAGAGAGACCTAGTGACAGC
TATCTCCGTCAAGGTGCAGAAGGAGAGATCATTGAATGTAGCATTTTCATGCAAAAAAAAAAATGTTGAAGTCTTTGGAC
TTCGGGAGTCTGTCCAAACTGCAGGTCACTCAGCCTACAGTTGGGATGAATTTCAAAACACCAGTTGGAGCCGGTTGAAT
CTTTCTGCTATGCTGTAATATTTTCAGTAAACCCAGCGCAACAACAACAACAAAACACAAAAGGAGGAGAAGCAGCCAAG
TCTCTTGGTTTACAGAGTAGCTCCTAATACCCCTTGCTGTCTGTCTCAAGTGCCCAATGGGAAGATAGTCAAAACAATAT
TCACACCTGTGATTCATCTCTCTACATGCAGTGTGTGTGAATCTTTATATACTGCATATTAAGGATCTGTCTTTACAGAT
AAAAACTAAAGCATTGAAGGAACTCCTTGTTTTGACTTATCAAAGTCCTTAAGAAAATACTAGAAAATTATAGCCATTGT
TTCAAATTTTAGCTTTATATTATCACTTGAAATGTGATGAAATGTGGCTGATAGATAATAATTCACTGATAACCTACAGA
CAATTCCCATCTTAAAATGGACCATTGGATTGAAGAATTAAATAAAATTGAGGGTTTTCCTTACATGTTTTGTCTAAAGA
GCGAAGTAGAAACAACTGTTCATAGATCTTCATTGAGGATTCGCATGTGAAGTAAGTACTCCTAACATAAACAAGTGGAC
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AACATTCTTGAGGGCTGGAAAATCTGAATGTTAGGACACCAAATATCTCCAGAAAACAAGTTTTATATTTCTAATCCTGC
ATAATAAACCTGGGGCCACTGCAGGCCTCATTAATAAAAACCTAATGGTATAACAATAATGAGGAGGAAATGCCAATGCC
GCACAAATCTGTTGAGACTAAAATATTTCTCACCCCAGCAGGCTTGGTGCATTTGACACTTCATGATATCAGCCAAAGTG
GAACTAAAAACAGCTCCTGGAAGAGGACTATGACATCATCAGGTTGGGAGTCTCCAGGGACAGCGGACCCTTTGGAAAAG
GACTAGAAAGTGTGAAATCTATTAGTCTTCGATATGAAATTCTCTGTCTCTGTCAAAAGCATTTCATATTTACAAGACAC
AGGCCTACTCCTAGGGCAGCAAAAAGTGGCAACAGGCAAGCAGAGGGAAAAGAGATCATGAGGCATTTCAGAGTGCACTG

FIG. 14(cont'd-2)

TCTTTTCATATATTTCTCAATGCCGTATGTTTGGTTTTATTTTGGCCAAGCATAACAATCTGCTCAAGAAAAAAAAATCT
GGAGAAAACAAAGGTGCCTTTGCCAATGTTATGTTTCTTTTTGACAAGCCCTGAGATTTCTGAGGGGAATTCACATAAAT
GGGATCAGGTCATTCATTTACGTTGTGTGCAAATATGATTTAAAGATACAACCTTTGCAGAGAGCATGCTTTCCTAAGGG
TAGGCACGTGGAGGACTAAGGGTAAAGCATTCTTCAAGAATCAGTTAATCAAAGAAAGGTGCTCTTTGCATTCTGAAATG
CCCTTGTTGCAAATATTGGTTATATTGATTAAATTTACACTTAATGGAAACAACCTTTAACTTACAGATGAACAAACCCA
CAAAAGCAAAAAAGCAAAAGCCCGACCTATGATTTCATATTTTCTGTGTAACTGGATTAAAGGATTCCTGCTTGCTTTTG
GGCATAAATGATAATGGAATATTTCCAGGTATTGTTTAAAATGAGGGCCCATCTACAAATTCTTAGCAATACTTTGGATA
ATTCTAAAATTCAGCTGGACATTGTCTAATTGTTTTTTATATACATCTTTGCTAGAATTTCAAATTTTAAGTATGTGAAT
TTAGTTAATTAGCTGTGCTGATCAATTCAAAAACATTACTTTCCTAAATTTTAGACTATGAAGGTCATAAATTCAACAAA
TATATCTACACATACAATTATAGATTGTTTTTCATTATAATGTCTTCATCTTAACAGAATTGTCTTTGTGATTGTTTTTA
GAAAACTGAGAGTTTTAATTCATAATTACGTTGATCAAAAAATTGTGGGAACAATCCAGCATTAATTGTATGTGATTGTT
TTTATGTACATAAGGAGTCTTAAGCTTGGTGCCTTGAAGTCTTTTGTACTTAGTCCCATGTTTAAAATTACTACTTTATA
TCTAAAGCATTTATGTTTTTCAATTCAATTTACATGATGCTAATTATGGCAATTATAACAAATATTAAAGATTTCGAAAT
AGAATATGTGAATTGTTCACCATACATAGAAATGAAAAGTTCATTTCGTAAAGCAAGATGCTGGGTGAAAGAGTGCTTTT
GATTGAAAGATCACTAGATTAGTAGAGGGCAAGACTTTTAGTCCCTAATCTACCCTTAATAGCCATGTGGTCACGTGTAA
GTCAGTGAACCCATCTCATTCTCCTCATACTTTTTTCATCTCTAAAATGAGGGTATAATTTAAGCTCGTTCATTTTTTTT
TTTTTTTGAGATAGAGTTTTGCTCTTGTCACCCAGGTTGGAGTGCAATGGCACGATCTCAGCTCACTGCAACCCTCTGCT
TCCTCGGTTCAAGTGATTCTCCCTGCTTCAGCCTCCCAAGTGAGCCCGGGATTACAGGTGCCCGCCACCACATCTGGGCC
TAGATTTTTTGTATTTTCACCATGTTGGCCAGGCTGGTCTCGAACCCCTACCTCAGGTGATCCCTCGCCTCGGCCTCTCA
AAGTGCTGGGATTACAGGTGTGAGCCACCACGCCCAGCCCAATATCAGTTTTTCTTTTTTAACACAAGGCTAACACAATC
AAAATACTAGCTAGGGGAGAAAAAAAAAATAAGGCACTGTTTATGTGTAACAGGCTCTTGTTGCAATCCACTGGGGCAGA
CCAAATAAACAGTAAGAATCAAATCCTTTTCATATAATCCTTTCTTTGCAGAATACATAAAATCCCCACAAATGGCTTAT
CTTCCTTTTTATGATATGTTGGAGAATTGTAGCTAAGTGACAGATATTTTGCTTGGGTGTATAGACCACAAAGGACTGTG
TCTTGATGATGGTTTGCATAAAATTATACCTTAGTTTTTACTTTGTATGTTACATGTTAGATTTAGAGTATGAAAATTAG
TAGGGAGGATTATTAACAAAGAACAGGGCAAGAGGAGTAGAATTAAACCTCTTCTAATACCTGTGCACAAGTAGGCTTTT
CAGAAACTCTACAACCCCAACATAAACTGGATAGTTAGAAAAGCACACTCCCAAGGAAGGCGGTTATGTTTTGCAGTTTG
AATCAGAAGAATAGAGCTATAGCAATCTTCATTCTATAGTAACATTAAAGAGCCTGGTTTATATTATAGCAGTCATTAAG
ATTTAAAAATTTACATCTTGCCGTTCTTCTTACTCACAGATTTTCGAGAGGTAATGTAATGATCACACGAGGTGAGAATC
ACTGCCTTTTATAATGCGATTAAATGCATGAACAAAGTTTCCAACAAATAACAGTAATAAAAAGAAACATGTATTAGCAC
TTAATAAGCCAGGTGCTGTACGACGTGTGTTACATGCTTTCAATCCATGAACTGGTAAACTGGTACTAGTATCTCTATTG
GACATGTGAGGAAACCAAATGGAGTTGATAAACAGTAGAGTTAAAAATTACTCTTCATATATTATATTGCCTCAATCTCA
CAGACATCTCTGCTACCAAAAGCTATCATATCTAGACTCGA

FIG. 14(cont'd-3)

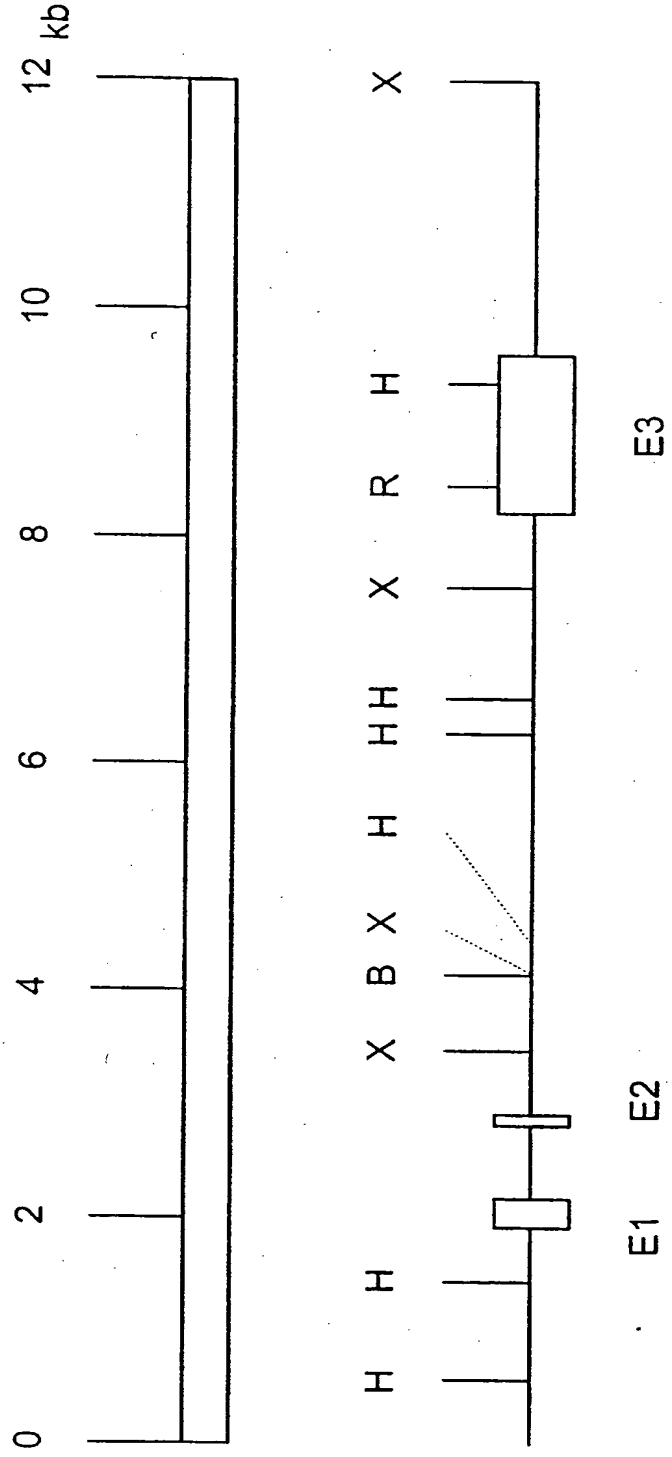

FIG. 15

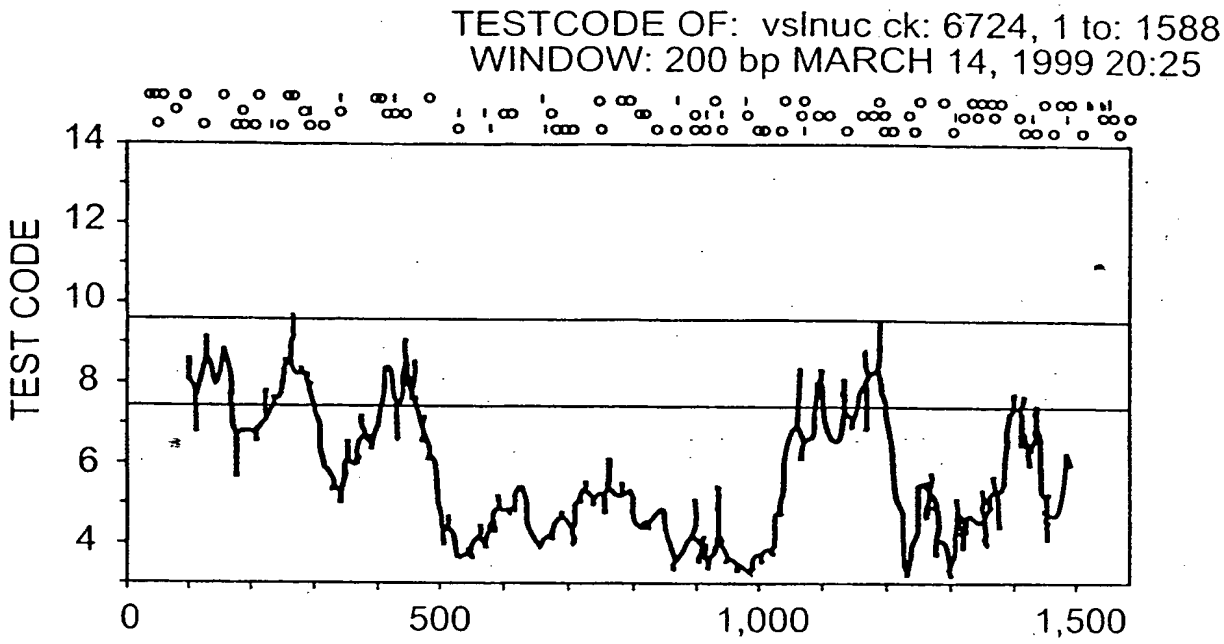

FIG. 16A

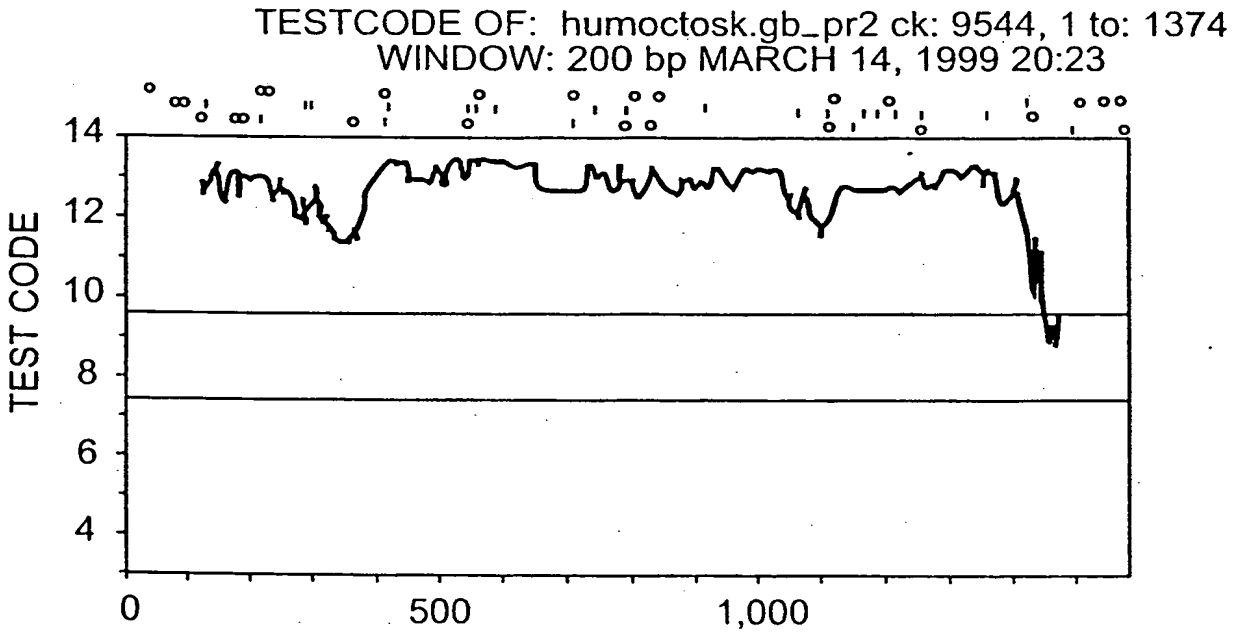

FIG. 16B

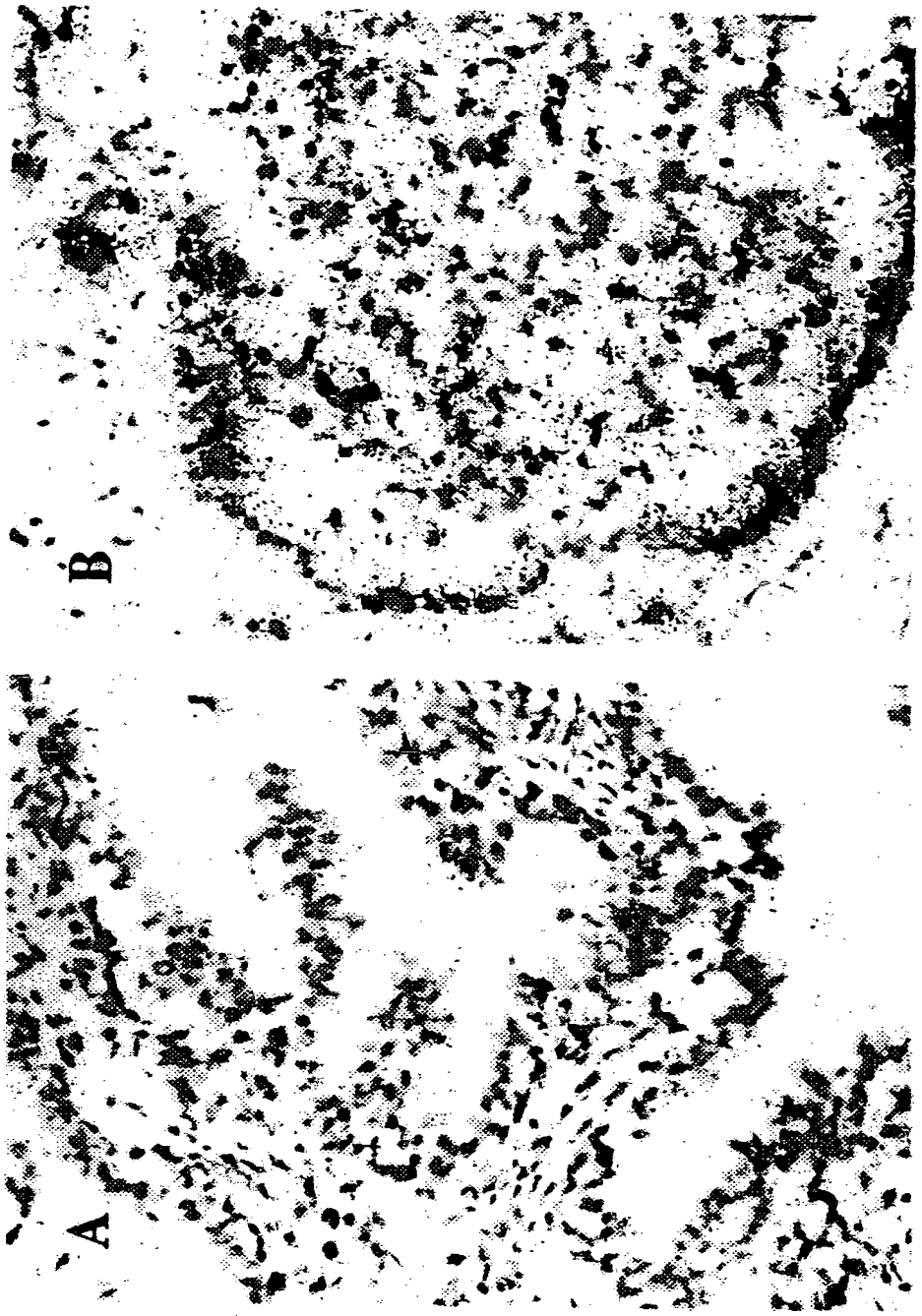

FIG. 17

SEQUENCE LISTING

<110> Srikantan, Vasantha
Zou, Zhiqiang
Moul, Judd W.
Srivastava, Shiv

<120> PROSTATE-SPECIFIC GENE, PCGEM1, AND METHODS OF USING PCGEM1 TO DETECT, TREAT, AND PREVENT PROSTATE CANCER

<130> 4995.0053-003-04

<140>
<141>

<150> 60/126,469
<151> 1999-03-26

<160> 22

<170> PatentIn Ver. 2.1

<210> 1
<211> 1603
<212> DNA
<213> Homo sapiens

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atccaaagtg gaactaaaaa cagctcctgg aagaggacta tgacatcatc aggttgggag 360
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tagggcagca aaaagtggca acaggcaagc agagggaaaa gagatcatga ggcatttcag 540
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<223> Description of Artificial Sequence:Probe/Primer

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<210> 6
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<220>
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<210> 7
<211> 19
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<213> Artificial Sequence

<220>
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<223> Y may represent any of the four nucleotide bases

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INTERNATIONAL SEARCH REPORT

Internr al Application No
PCT/US 00/07906

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C12N15/11 C12N9/00 C12Q1/68 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	"AC AC003046" EBI DATABASE, XP002143197 the whole document ---	5
X	SRIKANTAN V ET AL: "Structure and expression of a novel prostate specific gene: PC-GEM1." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL, vol. 40, March 1999 (1999-03), page 37 XP000929230 90th Annual Meeting of the American Association for Cancer Research;Philadelphia, Pennsylvania, USA; April 10-14, 1999, March, 1999 ISSN: 0197-016X abstract --- -/--	1-7,10, 11

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 July 2000	07/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kania, T

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INTERNATIONAL SEARCH REPORT

Internr al Application No
PCT/US 00/07906

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 99 00498 A (HUMAN GENOME SCIENCES INC ;HE WEI WU (US); CARTER KENNETH C (US)) 7 January 1999 (1999-01-07) the whole document	1-17
A	BUSSEMAKERS M J G ET AL: "A NEW PROSTATE-SPECIFIC MARKER, STRONGLY OVEREXPRESSED IN PROSTATIC TUMORS" UROLOGICAL RESEARCH,DE,SPRINGER VERLAG, BERLIN, vol. 25, no. 1, 1 February 1997 (1997-02-01), page 76 XP002074305 ISSN: 0300-5623 abstract	1-17
A	WO 95 19434 A (CALYDON INC) 20 July 1995 (1995-07-20) the whole document	12-15
A	WANG ZHOU ET AL: "Genes regulated by androgen in the rat ventral prostate." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 94, no. 24, 25 November 1997 (1997-11-25), pages 12999-13004, XP002143199 Nov. 25, 1997 ISSN: 0027-8424 the whole document	16
P,X	"AC AC013401" EBI DATABASE, XP002143200 the whole document	1-5

INTERNATIONAL SEARCH REPORT

ormation on patent family members

Interna al Application No
PCT/US 00/07906

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9900498 A	07-01-1999	EP 0996725 A	03-05-2000
WO 9519434 A	20-07-1995	US 5830686 A	03-11-1998
		AU 692837 B	18-06-1998
		AU 1686995 A	01-08-1995
		CA 2181073 A	20-07-1995
		EP 0755443 A	29-01-1997
		JP 9509049 T	16-09-1997
		US 5648478 A	15-07-1997
		US 6057299 A	02-05-2000